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The Potential Effects of Hydrogen Sulfide Gas from Geothermal Energy Conversion on Two Plant Species Native to Northern New Mexico

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ABSTRACT

THE POTENTIAL EFFECTS OF HYDROGEN SULFIDE GAS FROM GEOTHERMAL ENERGY CONVERSION ON TWO PLANT SPECIES NATIVE TO NORTHERN NEW MEXICO

BY

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Dry weight of topgrowth, water content of topgrowth, leaf nitrogen content, and leaf chlorophyll content were measured in well-watered, field-exposed little bluestem (Schizachyrium scoparium Nash.) and mountain brome (Bromus marginatus Nees.) plants fumigated with various mean levels of H_2S ranging from 0.05 to 3.58 ppm. The youngest fully expanded leaves were sampled for chlorophyll content after 60, 80, 100, and 140 and 60, 80, 120, and 140 h total of fumigation for little bluestem and mountain brome, respectively. All other responses were measured after 140 h total of fumigation.

The plants received a 7-day fumigation-free period prior to the seventh week (140 h) of fumigations.

Dry weight of little bluestem plants which received low concentrations of H_2S (0.11 ppm) increased by 94% of the control. Dry weight of little bluestem plants which received higher concentrations of H_2S (0.12 to 0.48 ppm) was reduced to the control level. At the highest H_2S concentration (2.39 ppm) dry weight of little bluestem was reduced by 44% of the control. There was no evidence that the reduction in nitrogen content (38%) at low H_2S levels ($\leq$ 0.11 ppm) was detrimental to plant growth. The productivity increase at these low concentrations may have been partially due to increases in leaf chlorophyll content (28%) and decreases in water content (16%). The linear dependence of dry weight on leaf chlorophyll content diminished as H_2S stress increased. The productivity increase may have partially reflected the usage of sulfur from H_2S as a nutrient source.

Mountain brome was relatively unaffected at the different concentrations of H_2S until 3.58 ppm H_2S was received where dry weight was reduced by 37% of the control.

There was evidence that changes in leaf chlorophyll content as total exposure time increased was partially due to air temperature changes. The decline of leaf chlorophyll content for both little bluestem and mountain brome appeared to be elastic strain because fumigated plants recovered to control or above-control levels. The mechanisms behind H_2S-caused stimulation or inhibition of chlorophyll synthesis or destruction of chlorophyll are complex.

INTRODUCTION

Background

Geothermal energy, although not benign, is a desirable alternative to dependency upon other finite and relatively more environmentally harmful energy conversion sources (Joyce and Fontes, 1978). However, as with most energy technology developments, environmental effects must be anticipated.

One area of potential geothermal development is within the Valles Caldera at the Baca Location about 30 km west of Los Alamos, New Mexico (Fig. 1) where a considerable amount of exploration has already occurred. The Baca Known Geothermal Resource Area (KGRA) has been estimated by the Department of Energy (1979) to have a potential electric generation capacity of approximately 400 megawatts. In 1978 Union Oil Company, the Public Service Company of New Mexico, and the Department of Energy announced their plans to construct a demonstration 50-megawatt, liquid-dominated, hydrothermal power plant at the Baca Location (U.S.D.O.E., 1979). Since then, they have discontinued the project and have placed the geothermal energy exploration and development rights up for bid.

A major concern at many existing hydrothermal power plants is the release of hydrogen sulfide (H_2S) gas into the surrounding region and its effects on vegetation. Similar problems will be encountered at most planned hydrothermal power plants (Anspaugh and Phelps, 1978).

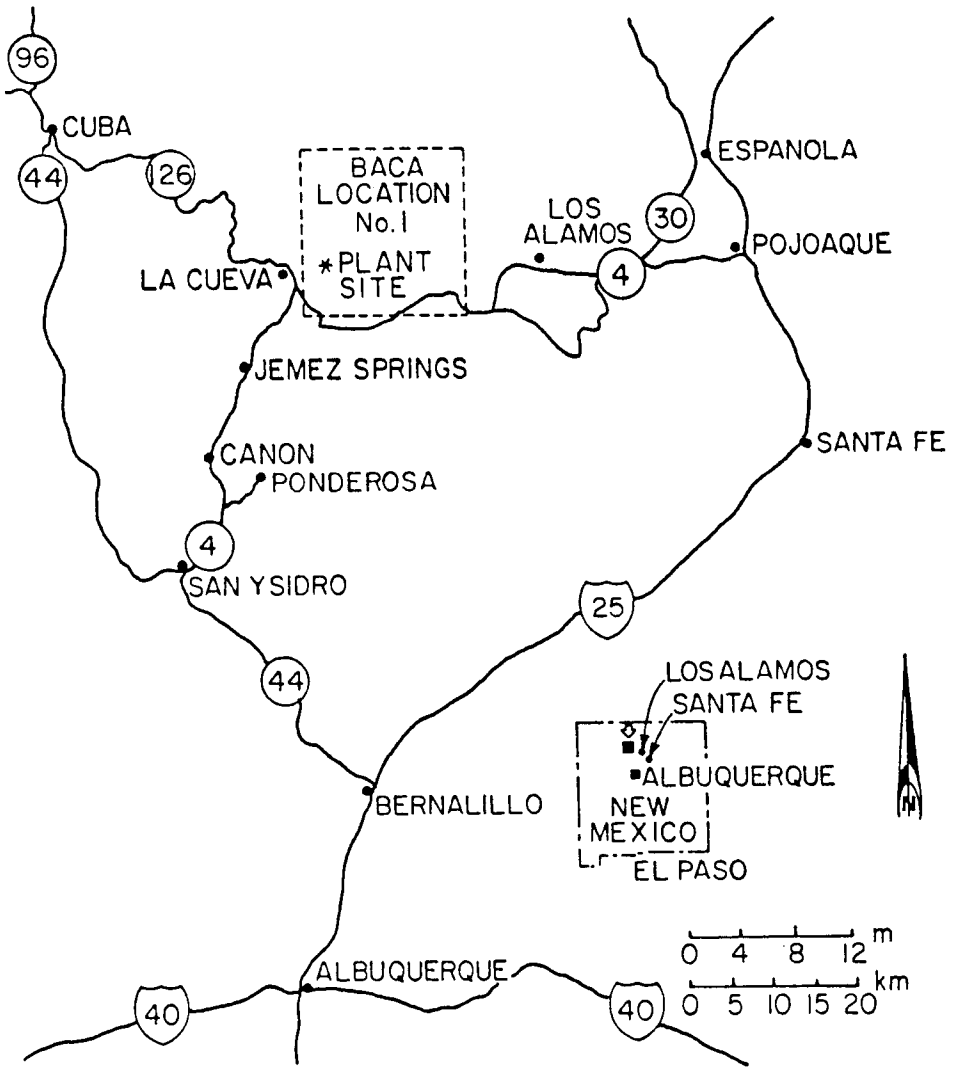

Fig. 1. Regional location of the Baca Location No. 1 Land Grant and the proposed plant site and surrounding well field. Source: Mountain West Research, Inc. Socioeconomic Analysis for the Proposed Baca Geothermal Project, 1979.

H_2S is a noncondensible gas (Joyce and Fontes, 1978) associated with geothermal steam, which, if permitted to collect in low or confined areas, can be lethal to plants (Axtmann, 1975). However, H_2S can also produce stimulatory effects on plant growth (Shinn et al., 1977) which may be beneficial in many areas.

To a large extent, H_2S will be removed from the Baca Geothermal Fluid by an abatement process; however, the exact amount is questionable. The Department of Energy (1979) predicted that after H_2S abatement, normal power plant operation would result in atmospheric H_2S levels of approximately 2 parts per billion (ppb) at the Baca Boundary. Joyce and Fontes (1978) reported that, of the H_2S abatement technologies available at the Geysers, only one was in full-scale operation, and with unsatisfactory results. Thus, significant amounts of H_2S are typically contained in the noncondensible gases released by hydrothermal power plants (Anspaugh and Phelps, 1978).

Sterns-Roger Incorporated (1979) projected that the potential highest 1-h average ground-level H_2S concentration within the Baca KGRA would be about 83 ppb. However, in an earlier report Gonzales (1980) discussed the possibility that expansion of power plant capabilities and contributions of naturally occurring H_2S could result in ground-level H_2S concentrations of 0.26 parts per million (ppm) and also, that temperature inversions, confinement pockets, and abatement system failure could elevate the estimated concentration even further.

These concentration levels have produced significant measurable effects on crop and forest plants (Shinn et al., 1976; Thompson and Kats, 1978; Coyne and Bingham, 1978). However, plant species differ widely in susceptibility to H_2S (Mudd, 1979) depending on genetic variability (Bradshaw, 1976), environmental interactions (Omrod, 1978) and other factors.

From an economic standpoint, the use of geothermal energy and resultant releases of H_2S near natural vegetation may have impact not only on timber production as once thought but on other economic values including livestock carrying capacity, watershed protection, recreational use, wildlife habitat, species diversity, and aesthetic use (Bradshaw, 1976). The Valles Caldera Region certainly stands to be affected in one or all of these values through changes in native plants. Determination of the potential effects of H_2S on vegetation native to the prospective KGRA would allow estimations to be made of the potential for impact gain or loss incurred by the public. Thus, potential effects on vegetation and the resulting economic impact of geothermal sources should be determined before tapping such sources (Mudd, 1979).

Few authors have examined the long-term effects of wide ranges of H_2S concentrations on native species of plants under field conditions. Crop plants, which are used more commonly in fumigation studies than native species, may be less useful for developing the capacity to predict H_2S resistance because "crop plants generally have a narrow range of stomatal conductance values which is the result of being adapted to a continual supply of water" (Winner and

Mooney, 1980, p. 290). Thus, the experimental utilization of native species will yield important predictive capabilities including (1) the determination of the tolerance levels of different native species to different levels of H_2S; (2) input to the critical review of air quality standards; (3) input to considerations of the evaluation of H_2S control strategies and abatement systems; and (4) input to the evaluation of potential H_2S impacts based upon changes in H_2S emissions (Winner and Mooney, 1980).

Given this situation there is a need for further study of the effects of H_2S on native vegetation. Reporting the results of such a study may help to alleviate some of the recent opposition to geothermal development in the Baca KGRA and may contribute to the development of a geothermal program that would allow a compromise among the energy industries, other economic sectors, and the public. This study supports this concept through the pursuit of the objectives discussed below.

Study Objectives

The overall objective of this study was to determine the responses of the native grass species little bluestem (*Schizachyrium scoparium* Nash.) and mountain brome (*Bromus marginatus* Nees.) to controlled gradients of H_2S under field ambient environmental conditions. Specific objectives were as follows:

1. To examine the effects of several concentrations of H_2S on dry weight of topgrowth, leaf chlorophyll content, water

content of topgrowth, and leaf nitrogen content of little bluestem and mountain brome.

2. To establish dose/response relationships for impact to little bluestem and mountain brome dry matter production over a range of H_2S concentrations.
3. To determine the relationship of plant response variables in responses of these plants to H_2S fumigations.
4. To examine the implications derived from the above three in order to provide input into the evaluation of H_2S abatement systems, air quality standards, and potential H_2S impacts based upon changes in H_2S emissions for the Baca KGRA and possibly other KGRA's.

The Experimental Area

The experimental area was located about 23 km east of the proposed hydrothermal power plant site, on Los Alamos National Laboratory (LANL) property. Ideally, the experimental area would have been located slightly southeast of the proposed power plant area to take advantage of downwind ambient environmental conditions; however, access to that area was not possible. Numerous factors were considered in selecting the experimental area, including geographic and environmental setting as compared to those surrounding the proposed power plant site, distance to LANL for obtaining supplies, protection from vandalism, and accessibility.

The experimental area was located at an altitude of about 2,400 m. Ponderosa pine (Pinus ponderosa Dougl.) dominated the

overstory and Arizona fescue (Festuca arizonica Vasey) and little bluestem (Schizachyrium scoparium Nash.) dominated the understory. The vegetation surrounding the proposed power plant site was typical of the ponderosa pine habitat, as described by the Department of Energy (1979) in the Baca Environmental Impact Statement.

LITERATURE REVIEW

Introduction

Until recently few detailed impact or potential impact analyses of geothermal gas emissions have appeared as published literature. However, increasing interest in geothermal energy has encouraged scientists to study the effects of noncondensible geothermal gas emissions, especially H_2S, on various aspects of vegetation. Several investigators have examined the short-term effects of single H_2S concentrations on vegetation under laboratory conditions. However, in the English-language literature, there are no satisfactory studies on the long-term (> 30 days) effects of a wide range of realistic H_2S levels on vegetation under field conditions. A vast amount of research has been applied to SO_2 effects, and although some of the principles and effects of SO_2 are similar to that of H_2S, the actions of H_2S on plants may be quite different. For example, Steubing and Jäger (1978) reported that fumigation with 2.5 mg/m^3 (2.1 ppm) H_2S produced necrosis on Pisum sativum leaves which, in the early stages of symptom development, were significantly different from those of SO_2-fumigated plants. Thus, it is necessary to understand the mechanism of plant response to realistic levels of H_2S in order to make accurate predictions of change in plant and animal communities exposed to H_2S.

Chemical Behavior of H_2S in the Atmosphere

The chemical behavior of H_2S in the atmosphere was reviewed by Sprung (1976) and Thompson (1976). This literature showed that H_2S is converted to SO_2 through the intermediates of hydrosulfide anion (HS^-) and sulfur oxide (SO). Although H_2S reacts at a very "slow" rate with O_2, it reacts with OH^- at rates 1000 times faster than with other atmospheric gas species to form HS^-. The hydrosulfide anion then reacts with O_2 to form SO which reacts with O_2 or NO_2 to yield SO_2. In summary the chemical reaction scheme of H_2S in the troposphere is represented by the following:

$$H_2S \xrightarrow{OH^-} HS^- \xrightarrow{O_2} SO \xrightarrow[\text{or } NO_2]{O_2} SO_2 \qquad (1)$$

Based on this reaction, Thompson (1976) showed how the residence times of H_2S, HS^-, and SO in the atmosphere can be calculated using concentrations and conversion rate constants. The upper limit for the residence time of H_2S in the troposphere was calculated to be 18 h, which also represented the mean tropospheric conversion time of H_2S to SO_2. The H_2S concentration used in the calculation was not given.

H_2S Uptake and Exchange by Plants

Stomates of typical plants prove to be nearly optimal for maximum gas or vapor diffusion (Salisbury and Ross, 1978). Thus, plants are ideally adapted for absorption of gas molecules including H_2S.

This supports the idea that undissociated gaseous H_2S may maintain a state of dynamic equilibrium with that at the air-plant interface much like CO_2 does (Unsworth et al., 1976). This is represented by the diagram in Fig. 2.

After H_2S enters the leaf through stomata it goes into solution inside the substomatal cavity. Water solutions of H_2S are not stable and become turbid quite rapidly (Hendrickson, 1979). Most of the H_2S taken up by plants is metabolized to sulfate (Thompson, 1976) through the intermediates of HS^-, $S^=$, SO_2, and $SO_4^=$. The chemical reaction scheme of H_2S in plant leaves is represented by the following:

$$H_2S \xrightarrow{OH^-} HS^- \xrightarrow{O_2} S^= \xrightarrow{O_2} SO_2 \xrightarrow{O_2} SO_4^= \quad . \qquad (2)$$

Dissociation of the first proton results in the formation of the hydrosulfide anion (HS^-). Dissociation of the second proton results in the formation of the sulfide anion ($S^=$). The sulfide anion then goes through a series of oxidations to yield $SO_4^=$. At the physiologic pH of 7.4, about one third of the total sulfide exists as the undissociated acid, about two thirds as the hydrosulfide anion, and a trace amount as the sulfide anion (Smith, 1979). At 18°C, the pK_a for the conversion of H_2S to HS^- is 7.04, whereas the pK_a for the conversion of HS^- to $S^=$ is 11.96 (Smith, 1979).

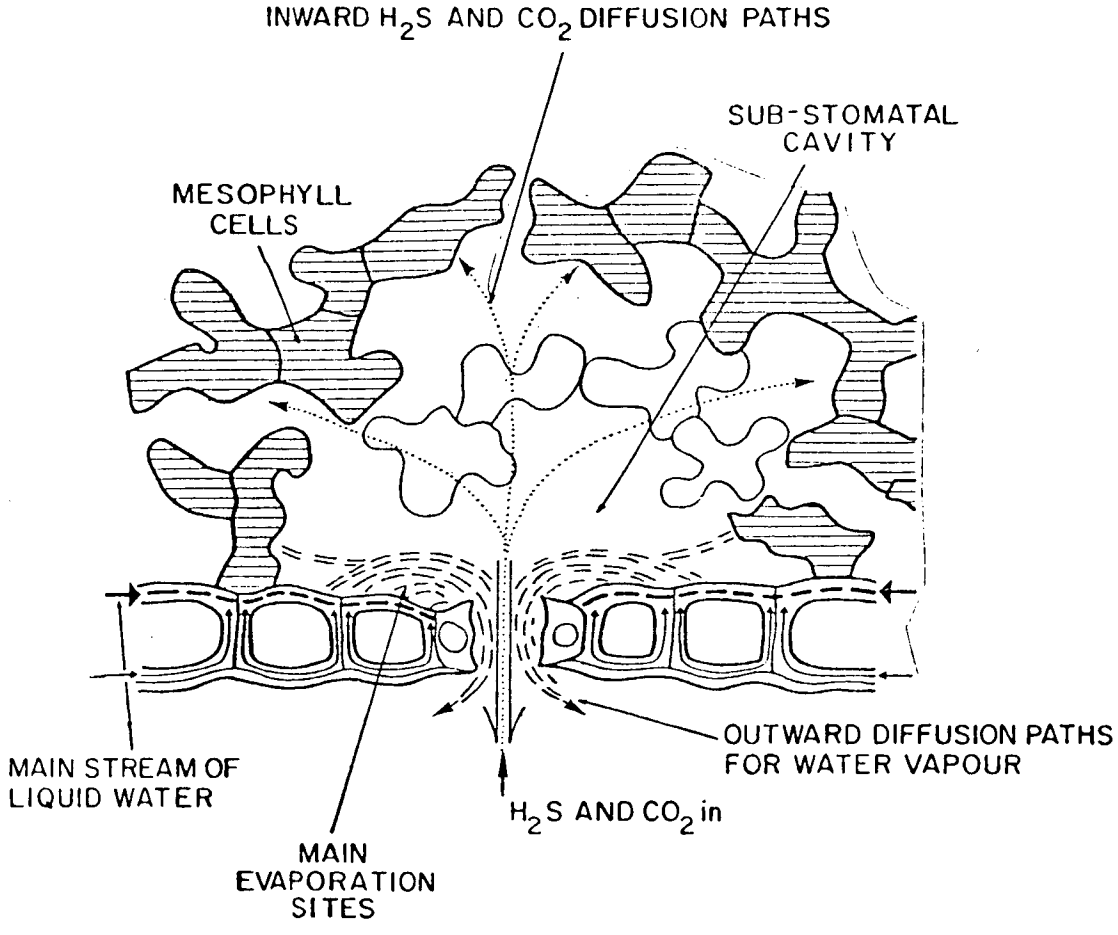

Fig. 2. Diagrammatic representation of gaseous H_2S and CO_2 uptake by a plant stoma. Source: Page 29 in Meidner, H. and D. W. Sheriff, 1976. Water and Plants. Blackie & Sons Limited.

H_2S Uptake and Plant Resistance

The properties discussed above are highly relevant to the effects of H_2S on plants. The literature vaguely indicates that the undissociated form (H_2S), which more readily penetrates biologic membranes, is the most toxic form to plants (Smith, 1979).

Taylor (1978) described the processes by which gaseous molecules enter plants and the components of plant resistance to the stress resulting from the presence of these molecules. A modification of his discussion will serve to explain H_2S uptake and resistance by plants.

Plants can close their stomata, thus reducing gas exchange and preventing the gas from reaching the cell surface within the intercellular leaf spaces (Mudd, 1975; Bell and Mudd, 1976; Unsworth et al., 1976; Taylor, 1978). Taylor (1978) called this process pollution stress avoidance in his revision of Levitt's (1972) ideas on air pollutant stress phenomena in plants. Stress tolerance is the other mechanism resulting in stress resistance. This resistance mechanism allows the plant to come to thermodynamic equilibrium with the H_2S stress by reparative or compensatory processes (Taylor, 1978).

Absorption of H_2S into the leaf interior, as shown in Fig. 2, must occur prior to the development of H_2S stress tolerance. The driving force for this uptake is molecular diffusion, while its rate is a function of the H_2S concentration gradient from the exterior to the leaf interior and the resistance to H_2S flow experienced by the H_2S molecules along the diffusion pathway (Taylor, 1978). A

change in either the concentration gradient or the resistance component alters the absorption rate of the H_2S (Taylor, 1978).

The concentration gradient results from a difference in H_2S concentrations between the leaf exterior, the leaf boundary layer, and the leaf interior (mainly the intercellular air space and the mesophyll cell surface) (Taylor, 1978). The extraction sites of H_2S molecules from the atmosphere by foliage are either internal (absorption) or external (adsorption) (Taylor, 1978). Leaf pubescence, surface water, and other plant features may provide effective sites for removal of H_2S molecules from the atmosphere (Taylor, 1978).

The main components of leaf resistance to H_2S flux are boundary layer, stomata, cuticles, and mesophyll cell surface. No further discussion of leaf resistance to H_2S will be made; however, excellent discussions can be found in works by Bennett et al. (1973), Unsworth et al. (1976), and Taylor (1978).

The ability of plants to exhibit H_2S stress avoidance, as defined by Taylor (1978), is not well documented, however, the fact that both internal and external factors, including gaseous pollutants, can alter epidermal resistance is well documented (Meidner and Mansfield, 1968; Taylor, 1978). At low H_2S concentrations (0.01 - 0.5 ppm), stomatal opening may actually be stimulated. At higher concentrations the uptake of H_2S and other air contaminants may be modified by conditions which cause stomatal diffusion resistance (Bennett, 1978; Coyne and Bingham, 1978;). When H_2S absorption has occurred, leaf response may be determined by the

internal H_2S concentration and by the biochemical threshold level of tolerance for H_2S and its toxic derivatives or dissociation products (Malhotra and Hocking, 1976). This resistance whereby the plant accommodates the stress without being killed is termed "pollutant stress tolerance" (Taylor, 1978). Leaf damage may occur if the internal H_2S concentration exceeds the threshold level (Taylor, 1978).

According to Taylor's (1978) discussion on the tolerance of plants to air pollution stress there are three mechanisms by which a plant can reduce or eliminate the harmful molecules of H_2S or its derivatives. These mechanisms are as follows: (1) the ability to continue metabolism unaltered despite the presence of the harmful molecules, i.e. tolerate; (2) the ability to convert H_2S and its derivatives to less harmful forms and to remove them to sinks; the chemical reaction of this process was discussed earlier; (3) the ability to maintain electrical neutrality by buffering the H_2S derivatives.

These types of hypotheses are not new. Ziegler (1972) proposed a hypothesis that C_3 and C_4 plants may differ in their susceptibilities to SO_2. This hypothesis was based on potential differences in the way the CO_2 carboxylating enzymes (RuBP carboxylase in C_3 plants and PEP carboxylase in C_4 plants) reacted to sulfite.

Responses of Higher Plants

The symptoms of injury caused by H_2S are generally confined to young foliage, with leaf margins being scorched. With less intense injury, interveinal portions of the leaf may show effects (Linzon, 1978).

McCallan et al. (1936) did some of the earliest significant works on the effects of H_2S on plant life by exposing selected species to very high concentrations (40 to 400 ppm) for periods that were as short as 2 to 4 h. They found that young plant tissue is generally more sensitive to H_2S than older tissue; injury symptoms were necrosis of young shoots and leaves, and basal and marginal necrosis of the youngest leaves; injury increased rapidly with increases in temperature; wilted plants were less sensitive than unwilted. The latter was attributed to increased stomatal diffusive resistance in the water-stressed plants. Until recently H_2S was considered as relatively nontoxic to plants because concentrations used were so much higher than suspected ambient levels and greater than levels of other highly injurious pollutants. However, Shinn and Kercher (1978) stated that under worst-case conditions, the threshold for injury (10% loss of leaf dry matter) is about 0.2 ppm H_2S assuming chronic exposure and no added CO_2 to ameliorate the effect.

Benedict and Breen (1955) also measured effects of H_2S (100 to 500 ppm) on several species. They found considerable species differences and greater susceptibility in younger tissue.

Thornton and Setterstrom (1940) found that the foliage of higher plants is more resistant to H_2S than to HCN, NH_3, Cl_2, and SO_2. No effect on the pH of the tissue exposed to H_2S was found.

Gassman (1973) reported that chlorophyll biosynthesis was inhibited by sulfide; however, the chemical mechanism of this effect was not understood. This effect was attributed to the possibility of H_2S acidification in which case lowering the pH caused the loss of Mg^{++} from the chlorophyll to form pheophytin. This is a well documented effect of SO_2 on chlorophyll (Mudd, 1975; Bell and Mudd, 1976).

Mudd (1979) reported results of Fallers' (1972) experiments in which young sunflowers (Helianthus annus L.) were exposed to H_2S fumigation while the plants had no alternate nutrient source of sulfur. The fumigations lasted 3 weeks. During this time the H_2S gas concentration varied between a few micrograms (ug/l) per liter and 280 ug/l (200 ppm). Both fresh and dry weights of the buds, the first five leaves, the stems, and the roots were measured. The plants exposed to H_2S were heavier in all respects than the controls, which were not supplied with sulfur in the nutrient solution. Analysis of the plants showed an accumulation of sulfur, especially in the roots. Faller (1972) thus demonstrated that H_2S can act as the sole source of sulfur for the nutrition of H. annus.

Shinn et al. (1976) examined the responses of field-grown lettuce to a synthesized geothermal gas mixture (15 CO_2 : 1 H_2S : 1 CH_4 : 2 N_2 parts by volume added to ambient air) over a 3-h period. They reported no significant depression of photosynthesis

until exposure concentrations approached 75 ppm CO_2 : 5 ppm H_2S added to air. This led them to conclude that CO_2 ameliorates the effect of H_2S acting alone. However, the variation experienced in photosynthesis was high (CV = 0.5). They found that low H_2S concentrations (1 ppm H_2S : 15 ppm CO_2 and lower) stimulated photosynthesis significantly more than controls and relaxed stomatal resistance.

In another study Shinn et al. (1977) exposed field-grown snap bean to a linear gradient concentration (2-7 ppm) of H_2S gas plus ozone for 4 h daily until 1 week before pod set. They reported a significant H_2S dose-response relationship despite the insensitivity of the plants to H_2S. They estimated that a dose of 6.42 ppm H_2S for 94.5 h was necessary to predict a dry matter production significantly lower than the controls at a 90% confidence level. "Low" concentrations of H_2S were reported to stimulate growth and increase photosynthesis. However, neither the gain nor loss in dry matter production could be interpreted as economic, which depended upon seed production.

Thompson and Kats (1978) reported that continuous fumigation of alfalfa (Medicago sativa L.), Thompson seedless grapes (Vitus vinifera L.), lettuce (Lactuca sativa L.), sugar beets (Beta vulgaris L.), California buckeye (Aesculus californica (Spach) Nutt.), ponderosa pine (Pinus ponderosa Laws.), and Douglas fir (Pseudotsuga menziesii Mirb.) with 3.0 ppm H_2S in greenhouses caused leaf lesions, defoliation, reduced growth, and death of sensitive species. Lesser, but similar effects, resulted with

0.3 ppm H_2S. Sulfur accumulation in leaves depended upon H_2S dosage. Faster growing plants accumulated sulfur more rapidly. Lower levels of H_2S, 0.03 ppm and sometimes 0.1 ppm, caused significant stimulation in growth of lettuce, sugar beets, and alfalfa. The stimulation occurred at certain times of the year and it was concluded that temperature and/or humidity could influence this.

In a sequal to this study Thompson (1976) conducted growth chamber studies by exposing pinto beans, hybrid sweet corn, and alfalfa to 0.6 ppm H_2S. When soil water was optimum, the pinto bean plants showed little leaf injury or reduced growth; however, "some" leaf injury occurred when water was withheld. This result is contrary to what one would expect based on the physiological mechanics and mechanisms of stomata. It is generally accepted that gaseous H_2S injury depends on entry through the stomata, and so conditions which favor open stomata at the time of exposure render the plants susceptible to injury. One explanation may be that stomatal opening was stimulated by the H_2S in the water-stressed plants. This concept is not new to those dealing with SO_2 effects on plants. Unsworth et al. (1972) reported that 0.1-0.5 ppm SO_2 stimulated stomatal opening, especially in water-stressed plants where stomata tended to be closed.

It becomes evident that the effects of gas contaminants on stomata are complex. Majernik and Mansfield (1972) reported a series of studies on the effects of SO_2 and other variables on stomatal opening. Although the responses of plants to H_2S are quite different than to SO_2 (Steubing and Jäger, 1978), the effects that

these gases have on stomata may be similar. Majernik and Mansfield (1971) emphasized that their findings were made entirely with Vicia faba, which may not be typical of other species. Mudd (1975) strengthens this by indicating that conditions used by Menser and Heggestad (1966), which caused the closure of tobacco stomata, would have caused opening of Vicia faba stomata.

In a study where Pisum sativum plants were fumigated with 2.1 ppm H_2S Steubing and Jäger (1978) reported that the H_2S fumigations produced water-stress conditions. This finding may have important consequences since we know that H_2S may cause stomatal opening in plants which were already water stressed prior to the fumigations. They attributed the accumulation of free proline to the water-stress conditions produced by the H_2S fumigation. The accumulation of large quantities of the amino acid proline in the plant vacuoles frequently occurs in halophytes (Salisbury and Ross, 1978). Another occurrence common to halophytes and reported on by Steubing and Jäger (1978) is an increase in osmotic pressure of the cell sap. Thus, the effects of excess H_2S uptake may produce effects similar to those seen in salt-stressed plants. Other plant responses reported by Steubing and Jäger (1978) were leaf necrosis, decreased fresh and dry plant weight and negative influences on water relations, photosynthesis, and respiration. Apparently, the activation of the plant enzymes glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) were stimulated by H_2S.

Coyne and Bingham (1978) reported the results of fumigating field-grown snap beans (Phaseolus vulgaris L.) with various levels of H_2S alone and in the presence of 0.072 ppm O_3 for 4 h each day beginning at the first trifoliate leaf stage. The results indicated a 25 and 10% increase in maximum stomatal conductance and maximum apparent photosynthesis, respectively, at 0.74 ppm H_2S. Higher concentrations depressed stomatal opening and CO_2 uptake, while O_3 plus H_2S depressed stomatal and photosynthetic response more than H_2S alone. They concluded that as pollutant stress increased, photosynthesis ceased to respond linearly to increasing stomatal conductance at lower conductance values indicating that mesophyll resistance to CO_2 transfer was more limiting than CO_2 diffusion through the stomata at higher light intensities.

Most of the studies cited here have been carried out with constant levels of H_2S. As Wellburn et al. (1976) disclosed, situations of intermittent and fluctuating levels of mixed pollutants are probably more common in realistic settings than the numerous single pollutant studies in the literature would imply. Although the study to be reported on in this paper did not deviate from the single pollutant studies, it did inadvertently depart from the steady-level studies by virtue of being conducted using open-top field fumigation chambers. Not to be misinterpreted though, the H_2S gas concentrations were reported in a constant-level fashion; however, intermittent and fluctuating levels were actually the case as caused by machinery malfunctions and wind, respectively. The variation from the constant-level concentrations was measured.

MATERIALS AND METHODS

Plant Growth and Care

Seeds of little bluestem (Schizachyrium scoparium Nash.), a C_4 species (Salisbury and Ross, 1978), and mountain brome (Bromus marginatus Nees.), a C_3 species (Salisbury and Ross, 1978), were planted in 25-cm diameter 15-cm deep plastic pots containing Terra-Lite Vermiculite. The mountain brome seeds were planted 5 weeks later than the little bluestem because of initial failures to establish a different plant (Festuca arizonica Vasey.) as the second species. Therefore, the mountain brome plants were always about 5 weeks behind the little bluestem plants in age. This required that sampling of the mountain brome plants take place 5 weeks later than that of the little bluestem plants in order to sample both species at about the same developmental stage. However, environmental weather variables were different for each species due to the time delay in establishing mountain brome. All plants were watered with a Miracle-gro Nutrient Solution prior to each fumigation, maintaining soils near saturation. No watering was attempted on "rain" days. Plants were thinned to about twenty plants per pot. No herbivore or weed problems were detected.

Experimental Procedures

Fumigations with H_2S were carried out in four rectangular, open-top chambers (Fig. 3) described by Gonzales (1980) and similar to those described by Coyne and Bingham (1978) and Shinn et al.

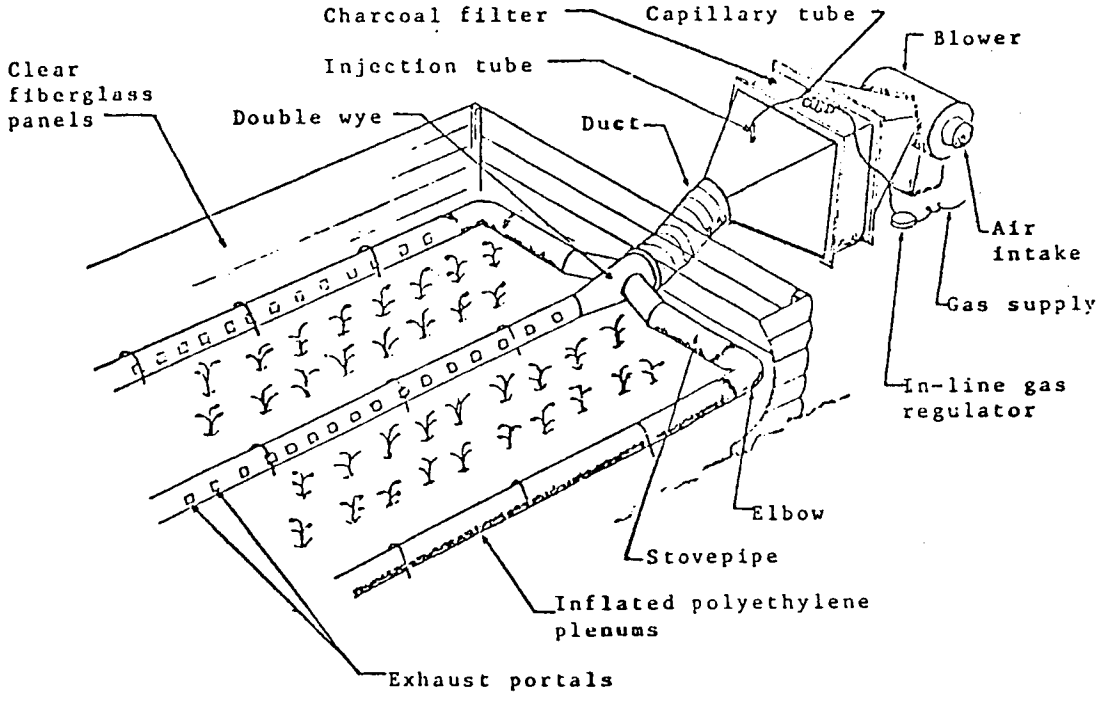

Fig. 3. An open top field-fumigation chamber for exposing plants to controlled levels of gases.

(1977). The H_2S delivery rates were fixed using stainless steel metering valves. A flow diagram can be seen in Fig. 4. Details of the chamber design, performance, and spatial distributions of injected gases can be found in works by Gonzales (1980) and Shinn et al. (1977).

The pots were arranged in twin rows on beds 1-m wide inside the chambers. There were two twin rows each of twelve or thirteen pots per row inside four rectangular minimum interference chambers 0.6 m high x 7.08 m long x 3 m wide. Thus, there were 25 pots of each plant species in each chamber totaling 50 pots per chamber. Each chamber contained a twin row of little bluestem and a twin row of mountain brome. Two of the four chambers were used to fumigate with H_2S gas and the other two were used as control chambers (no H_2S added). The spatial arrangement of the four chambers in relation to each other and in relation to vegetation in the experimental area is shown in Fig. 5. All pots were eventually buried so that only the upper 2.54 cm of the pot was above ground level. The purpose of this was to take advantage of less variation in H_2S gas concentrations near the ground surface (Gonzales, 1980).

Beginning at 2 weeks after emergence, the plants were placed inside the chambers and fumigated with H_2S plus charcoal-filtered air from 1000 to 1400 (MST) on Monday through Friday for a total of 7 treatment weeks (140 h) per species. Following the 6th week of fumigation of the little bluestem plants, which was concurrent with the 1st week of fumigation of the mountain brome plants, one blower-assembly motor malfunctioned. This provided an opportunity to

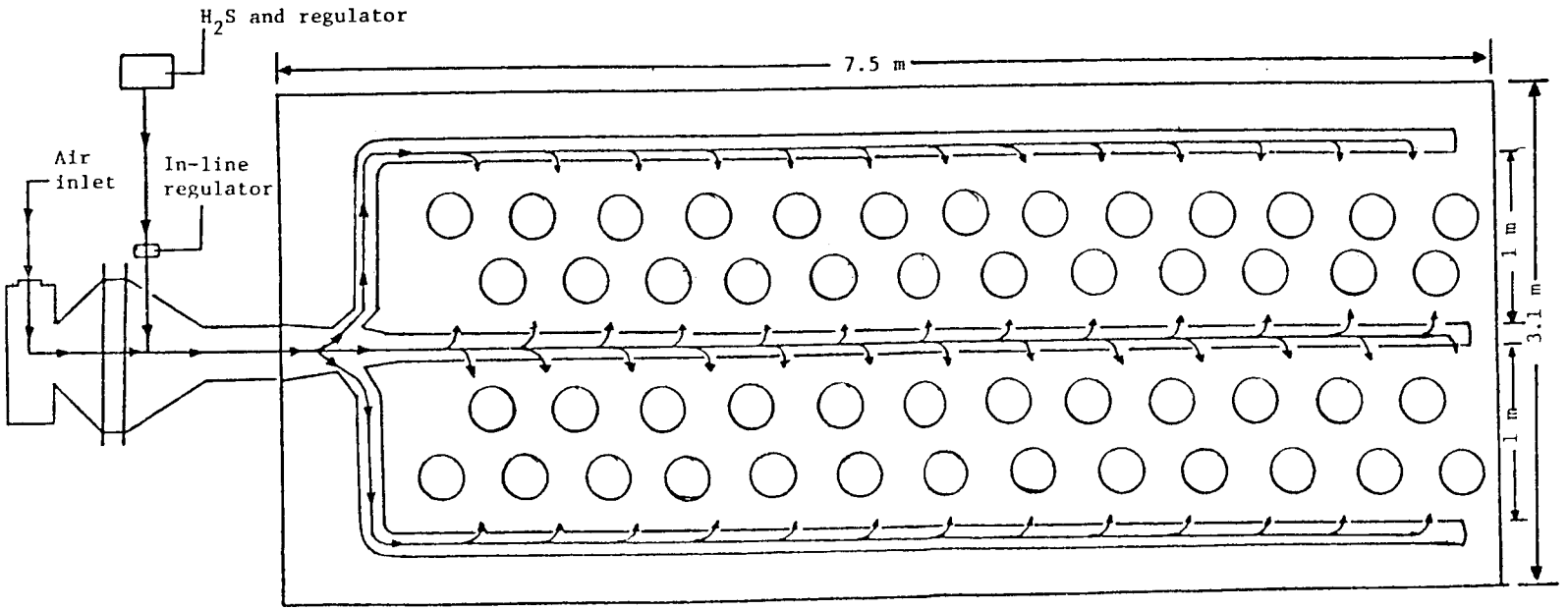

Fig. 4. A flow diagram of H_2S plus ambient air.

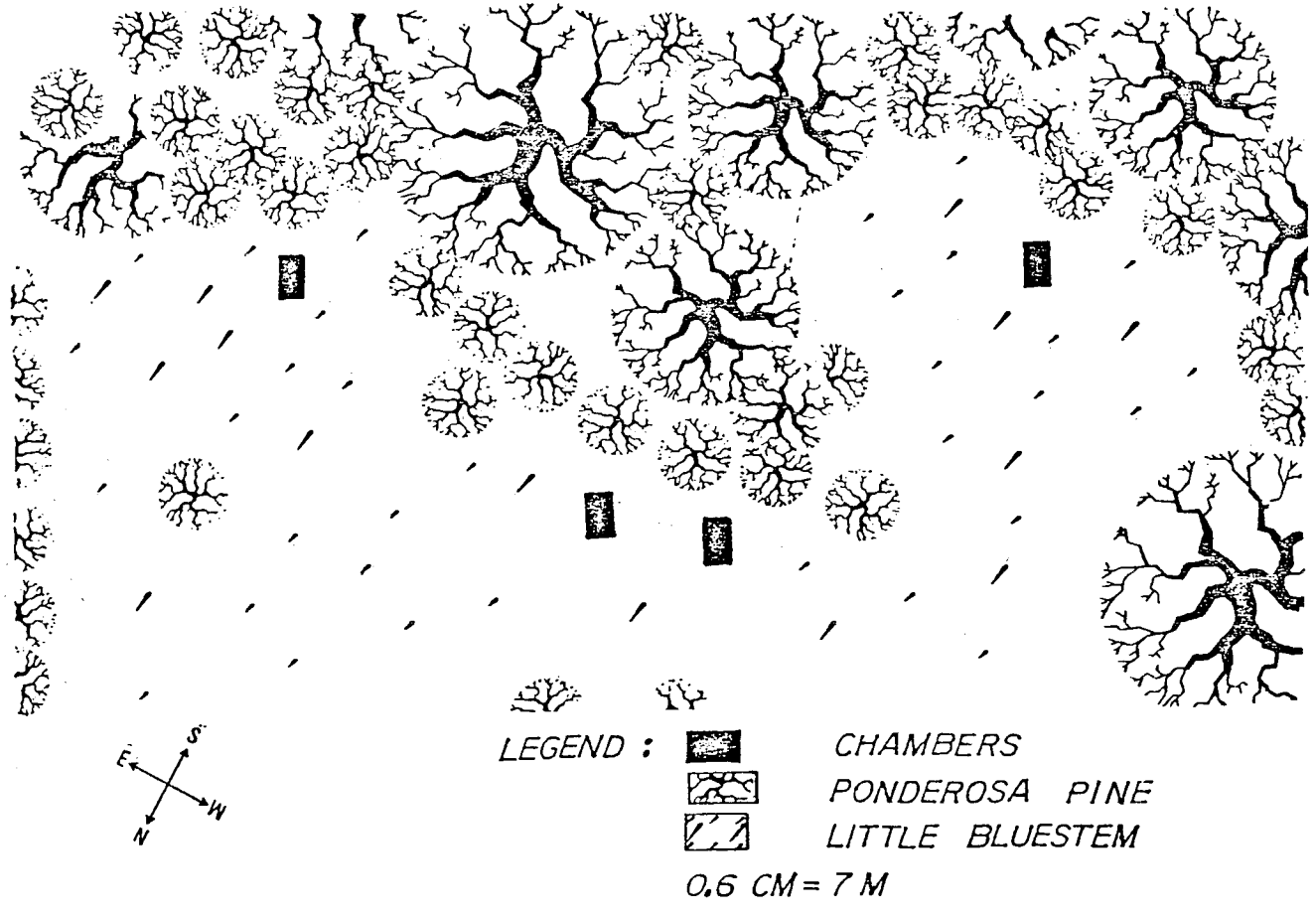

Fig. 5. The spatial arrangement of the 4 chambers in relations to each other and to vegetation in the experimental area.

evaluate the "recovery" capabilities of plants when given a fumigation-free period. However, in order to administer the fumigation-free period to both plant species at the same developmental stage H_2S treatments of the mountain brome plants were continued while the little bluestem plants received a 9-day fumigation-free period. This required the removal and rearrangement of pots so that only the mountain brome plants continued receiving fumigations during the 9 day period. During this time neither the control nor the H_2S-treated little bluestem plants received "treatments". Following the 9-day period (after the blower-assembly motor was repaired) all fumigation procedures returned to normal. The mountain brome plants were also given a 9 day "recovery" period when they reached the same developmental stage as were the little bluestem plants when they received their fumigation-free period. This second 9-day period occurred 12 weeks after fumigations had commenced. By then all the little bluestem plants had been harvested. Thus, the 7 weeks of fumigation to each plant species was completed in a 13 week period and there was only a three week overlap period during which both species were fumigated simultaneously. Control plants received charcoal-filtered air (0 ppm H_2S) concurrent with the H_2S fumigations. The range of H_2S concentrations in the "treatment" chambers over the duration of the study was 0.02 to 0.86 ppm in one of the fumigation chambers and 0.41 to 4.39 ppm in the other chamber.

One chamber was monitored continuously for H_2S by means of a flame-photometric total sulfur detector (Meloy Laboratories Inc., Model SA285E, 0-1 ppm). Daily spot measurements for H_2S were made

using a portable, fast-response, electrovoltametric detector (Intrascan Corporation, Model 1177) having a sensitivity of 2 percent at 1 ppm. Fumigations were carried out on schedule during rain storms often causing wide variations in H_2S concentations.

Although the chambers were designed to produce linear gradients of gas concentrations (Coyne and Bingham, 1978), H_2S gas concentration measurements were taken above pots and averaged for subplots as described by Gonzales (1980). Mean concentrations of H_2S for each subplot at the end of the experiment were 0 (control chambers), 0.05, 0.11, 0.22, 0.34, 0.51, 0.73, 1.16, 1.64, and 2.39 ppm for little bluestem plants and 0, 0.07, 0.21, 0.37, 0.65, 0.72, 1.16, 2.14, and 3.58 ppm for mountain brome plants. There was one more little bluestem subplot than mountain brome because of natural breaks in H_2S concentration along the length of the chamber. However, no changes were made after subplots were intially defined according to H_2S concentrations at the beginning of the experiment. Mountain brome plants were fumigated with higher H_2S concentrations than little bluestem plants because of hypotheses that mountain brome would exhibit greater H_2S stress resistance. This was achieved by varying the flow rate of the treatment air at the side stovepipes. Concentrations of H_2S were averaged to date after 60, 80, 100, 120, and 140 h-total of fumigation, therefore, the mean concentrations differed slightly from week to week. Coefficients of variation (CV) were calculated from the intermittent spot measurements taken over the entire range of temporal variability in ambient conditions (combined CV for H_2S measurements = 27%).

Air temperature, wind speed and direction, and relative humidity were recorded outside the chambers with a Meteorology Research Incorporated (MRI) weather recording instrument. Data on these and other environmental variables were also obtained from a LANL meteorology group.

Experimental Design and Statistical Analyses

The experimental design used was a restricted completely randomized design using the analysis of variance for any number of groups with unequal replication. The restriction was to keep species of the same kind together. Twenty experimental units (20 plants per pot) received unequal replication (a different number of pots per treatment) of each of 9 or 10 treatments (H_2S gas). Replication was achieved by grouping the pots into groups or subplots. Each subplot of mountain brome consisted of 6 pots with the exception of the subplot which received a mean H_2S concentration of 0.65 ppm which consisted of 7 pots. The 5 subplots of little bluestem in the "low" H_2S treatment chamber consisted of 5 pots each and the 4 subplots in the "high" H_2S treatment chamber consisted of 6 pots each. Because of the large variation expected among plants, two chambers were used as controls in order to reduce the CV in establishing a mean.

It was anticipted that the plants would respond differently even when treated alike; i.e., sources of variation other than treatment effects were anticipated. Thus, it seemed necessary to block the pots so that the portion of variability inherent in the plant could

be measured and excluded from the experimental error. However, there was no way to meaningfully group the pots of plants as described by Steel and Torrie (1960), so that groups would have some type of uniformity with respect to each other.

A one-way analysis of variance was used to check for significant differences between the experimental treatments of a species. More than one control mean (0 ppm H_2S) was often calculated (by row) to investigate significant differences between control treatments; this served as a way of checking for variation in measured plant responses caused by factors other than the H_2S treatment, especially chamber effect. Duncan's New Multiple Range Test (Steel and Torrie, 1960) was used to separate treatment means from each other statistically. Paired *t* tests were used to determine if there was a significant difference in the chlorophyll content of little bluestem plants before and after fumigations on a particular day during the 7th week of fumigation for that species.

Leaf Chlorophyll Content

Little bluestem leaves were sampled for chlorophyll content after 60, 80, 100, and 140 h total of fumigation as were mountain brome leaves after 60, 80, 120, and 140 h total. The sampling procedure at each of the sampling times was as follows: from each pot a young fully expanded leaf was removed from a randomly selected plant and taken to the laboratory for processing. Each leaf was then cut into small pieces and a 0.1 g sample was weighed. The only exception to this was after the 60 h-total fumigation time for mountain

brome when only the pots in the "low" H_2S-concentation chamber were sampled. Sample sizes (n) consisted of 5, 6, or 7 depending on the number of pots in each subplot as discussed under the "Experimental Design and Statistical Analyses" section. Sample sizes of "control" plants were much larger because two chambers were used as controls in order to examine the variability inherent in the plants.

Chlorophyll determinations were made by extracting the 0.1 g leaf tissue samples with 10 ml of aqueous 80% acetone in subdued to no light for 48 h with periodic shaking, following the methods of Vernon (1960). The absorbance of the extracts was measured at 649 and 665 nm by means of a Varian Techtron U.V.-V.S. model 635 scanning spectrophotometer using the 0.2 nm bandwidth-measuring beam and a 1-ml cuvette having a path length of 10 mm. Chlorophyll a, b, and total concentrations were calculated using the formula derived by Vernon (1960).

On a particular day, midway through the 7th week of fumigations, chlorophyll determinations were made on little bluestem extracts both before and after fumigations. This was done to determine the change in chlorophyll content which took place during the actual 4-h fumigation period. All other chlorophyll determinations were made after the 4-h fumigations on Fridays. Before-and-after chlorophyll determinations were made on H_2S-treated and control plant extracts to determine whether the daily change in chlorophyll content was due to the H_2S treatment or some other factor.

The absorbance measurements of the 7th-week little bluestem extracts were made in triplicate in order to determine whether the

variation contributed by the spectrophotometer and its use was a statistically significant source of sampling error. A two-way analysis of variance was used here to separate the sampling error from the experimental error and to check for significant differences between the experimental treatments.

Percent Water

At the end of the experiment (after 140 h-total of fumigation) plants were clipped at soil level and taken to the laboratory in preweighed plastic sampling bags. Plants were weighed using a Metler analytical balance. The percentage of the plant material comprised of water was calculated using the total fresh weights and dry weights of the above ground portion of the plants in each pot. The subplot sample size (n) was 5, 6, or 7 for the H_2S-treated plants of both species and 24 and 49 for the control plants of little bluestem and mountain brome, respectively.

Yield

After drying in a forced-air circulation oven at 80°C for 30 h the samples were reweighed. Average plant yield was determined by dividing the total above-ground plant dry weight per pot by the number of plants in that pot. Subplot sample sizes were 5, 6, or 7 for H_2S-treated plants and 48 total for the control plants.

Leaf Nitrogen Content

The dry plant material of each pot used for making yield determinations was powdered using a wig-l-bug pulverizer (Mfg. unknown). Percent-nitrogen determination were made by combusting preweighed 1-3 mg microsamples of the powdered plant material by means of a Perkin-Elmer 240B total combustion elemental analyzer. Sample sizes for all little bluestem subplots was 3. Subplot sample size for mountain brome was 5 or 6 for H_2S-treated subplots and 35 total for "control" plants.

RESULTS

Dry Weight of Topgrowth

Little Bluestem

The ANOVA table for dry weight of topgrowth data appears in Appendix Table 14. There was a highly significant difference in the treatment means; however, considering the magnitude of H_2S dosages used, the calculated F is lower than expected. The mean dry weight of little bluestem at several treatment levels is plotted in Fig. 6. Each dry weight data point and H_2S concentration represents the average of five or six pots (subplot) and the average of H_2S spot measurements taken in each subplot, respectively. Comparisons among treatment means using Duncan's New Multiple Range Test (Table 1) shows that the little bluestem plants fumigated with low levels of H_2S (0.05, 0.11, and 0.34 ppm) had a significantly higher mean yield than two of the control means at the 95% confidence level. Control means #1 and 2 and #3 and 4 did not differ significantly within chambers but showed a significant difference between chambers, implying that some source of variation other than treatment effects was present. Mean yield values of the higher concentration treatments (1.16, 1.64, and 2.39 ppm H_2S) were significantly less than two of the controls at the 95% confidence level.

The shape of the observed dose-response curve is shown in Fig. 6. At low concentrations there was an increase in dry weight

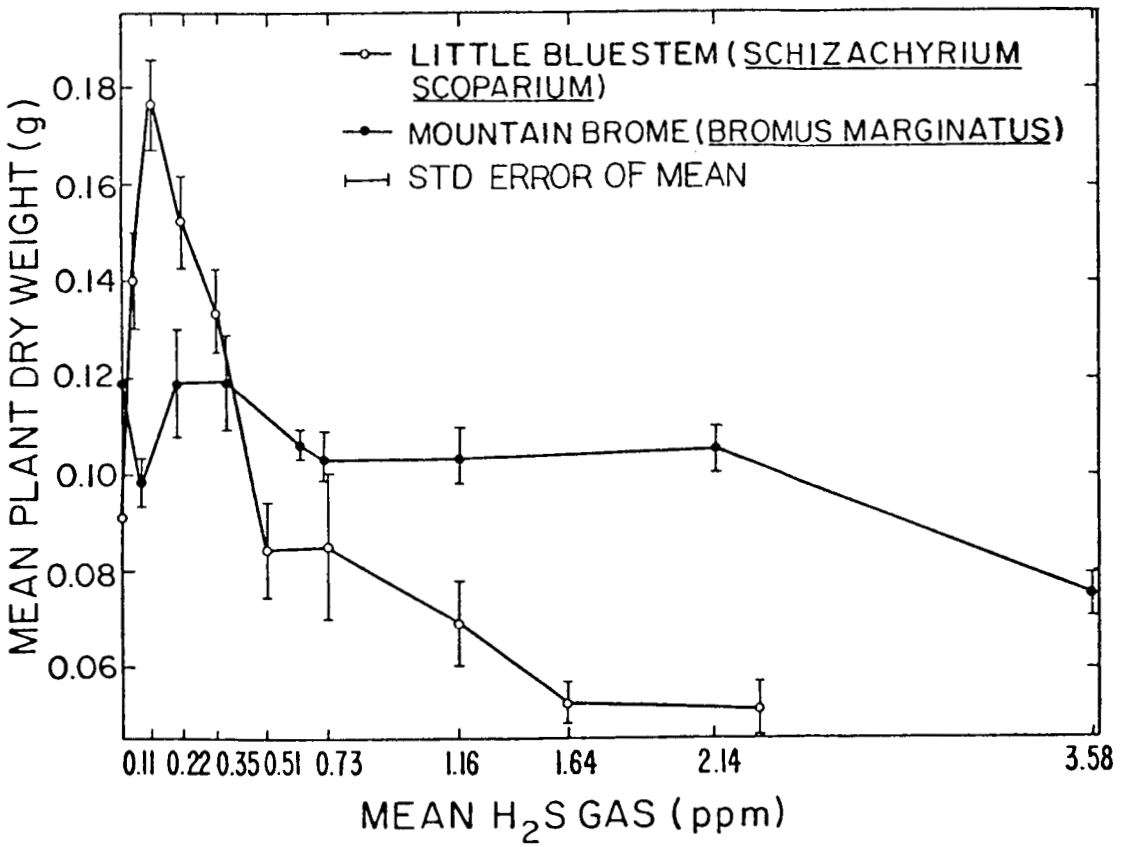

Fig. 6. Mean plant dry weight of mountain brome and little bluestem topgrowth after fumigations with H_2S for 140 h over an 8-week period.

Table 1. Comparisons among dry weight means of little bluestem plants using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means not underscored by the same line are significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean H_2S Conc. (ppm)	2.39	1.64	1.16	0	0	0.51	0.73	0	0	0.34	0.05	0.22	0.11
Mean Plant Dry Weight (g)	0.051	0.052	0.068	0.069	0.069	0.084	0.085	0.111	0.114	0.133	0.140	0.152	0.177

yield which can be interpreted as a stimulation of growth; as concentrations increased, there was a reduction in dry weight which can be interpreted as a depression of growth.

Plant dry weights of topgrowth were regressed on H_2S concentration. Regression curves (A and B) were fitted to the observed growth data of little bluestem (Fig. 7). For H_2S concentrations of 0.11 ppm and below (Curve A in Fig. 7) the correlation coefficient ($r = 0.97$) indicated a significant linear relationship at $\alpha = 0.05$ and for H_2S concentrations greater than 0.11 ppm (Curve B in Fig. 7) the correlation coefficient ($r = 0.76$) indicated a significant nonlinear relationship of the quadratic form at ($\alpha = 0.05$). The combined coefficients of determination for the regressions indicated that about three-fourths of the variation in the dry weight variable is explained by variation in the H_2S concentration. The regression of plant dry weight on H_2S concentration predicted a gain of 0.023 grams dry weight with each 0.03 ppm increase in concentration up to and including 0.11 ppm H_2S and a loss of 0.003 grams dry weight with each 0.03 ppm increase in concentration above 0.11 ppm H_2S. The regression equations for Curve A and B in Fig. 7 are $\hat{Y} = 0.091 + 0.77X$ and $\hat{Y} = 0.1483 + (-0.1033X) + 0.02691X^2$, respectively, where $\hat{Y}$ is the total dry matter in grams and X is the H_2S concentration in ppm.

Mountain Brome

The ANOVA table for dry weight of topgrowth data appears in Appendix Table 15. The dry weight of topgrowth data appears in

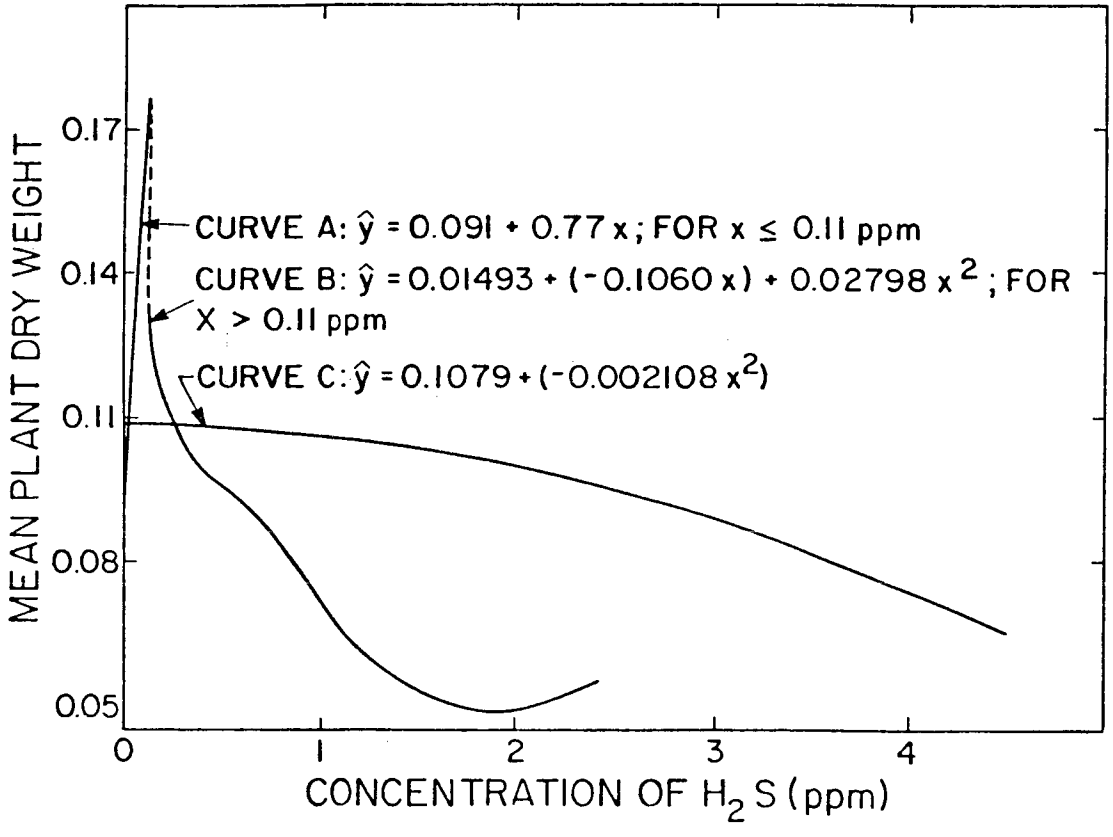

Fig. 7. Fitted regression curves for plant dry weight of topgrowth data of little bluestem (Curves A and B)and mountain brome (Curve C) after 140 h of fumigations with H_2S.

Table 2 and represents the average dry weight from six or seven groups of pots at nine levels of H_2S and the average of H_2S spot measurements taken in their respective subplots. The observed dose-response curve is plotted in Fig. 6.

A significant treatment effect was evident, however, it was difficult to ascertain any patterns by separating treatment effects using Duncan's New Multiple-Range test (Table 2). The treatment mean of plants fumigated with the highest H_2S level (3.58 ppm) was significantly different from the other treatment means except for the 0.07 ppm mean. Thus, dry weight of topgrowth of mountain brome plants may have been relatively unaffected by the H_2S treatments until concentrations reached 3.58 ppm H_2S, where there was a negative effect. The difference in control plant means between chambers for this species was about 16%. This difference is much lower than that observed for little bluestem, possibly indicating that the unknown extraneous source of variation affected little bluestem plants to a greater degree than it affected mountain brome plants.

Plant dry weight of topgrowth was regressed on H_2S concentration. A regression curve was fitted to the observed growth data of mountain brome (Curve C in Fig. 7). The correlation coefficient ($r = 0.55$) indicated a significant curvilinear relationship at $\alpha = 0.05$. The coefficient of determination for the regression indicated that only about one-third of the variation in the dry weight variable was explained by variation in the H_2S concentration using this regression equation. The regression of plant dry weight on

Table 2. Comparisons among dry weight means of mountain brome plants using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11	12
Mean H_2S Conc. (ppm)	3.58	0.07	1.16	0.72	2.14	0.65	0	0	0.37	0.21	0	0
Mean Plant Dry Weight (g)	0.075	0.098	0.103	0.103	0.105	0.106	0.109	0.109	0.119	0.119	0.129	0.131

H_2S concentration predicted a loss of 0.002 grams dry weight with each 1.0 ppm increase in concentration. The regression equation for Curve C in Fig. 7 is $\hat{Y} = 0.1079 + (-0.002108X^2)$.

Water Content of Topgrowth

Little Bluestem

The ANOVA table for water content of topgrowth data appears in Appendix Table 16. There was a significant difference in water content treatment means. The observed dose/response curve for water content means appears in Fig. 8. Comparisons among treatment means (Table 3) shows that the little bluestem plants fumigated with low levels of H_2S (0.05, 0.11, and 0.22 ppm) had a significantly lower mean water content than those fumigated with 0, 0.73, and 2.39 ppm H_2S. The reduction in water content at the low levels of H_2S may have resulted from two possible sources: (1) increased transpiration as caused by an H_2S-induced stimulation of stomatal opening similar to studies with SO_2 (Majernik and Mansfield, 1972; Unsworth et al., 1972) or (2) a process where the water potential in the plant apoplast goes below that of the protoplast resulting in water movement out of the protoplast.

The co-relation between water content and dry weight in their response to H_2S is shown in Fig. 9. There is a relatively strong ($r = 0.66$) negative relationship in the joint response of water content and dry weight to the H_2S fumigations. The lowered water

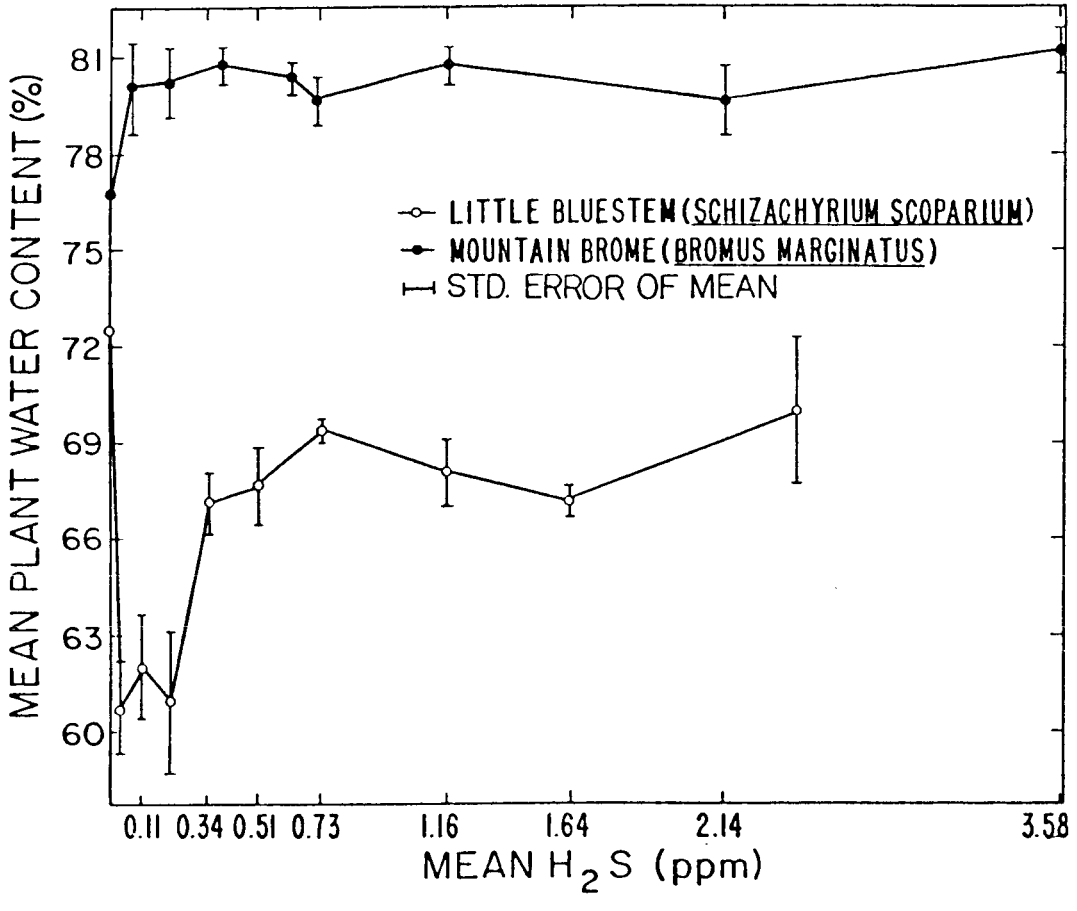

Fig. 8. Mean plant water content of little bluestem and mountain brome topgrowth after fumigations with H_2S for 140 h over an 8-week period.

Table 3. Comparisons among water content means of little bluestem plants using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level

Rank	1	2	3	4	5	6	7	8	9	10	11	12
Mean H_2S (ppm)	0.05	0.22	0.11	0.34	1.64	0.51	1.16	0.73	0	2.39	0	0
Mean Plant Water Content (%)	60.7	61.1	61.8	67.2	67.2	67.6	68.0	69.3	70.5	71.1	73.3	74.0

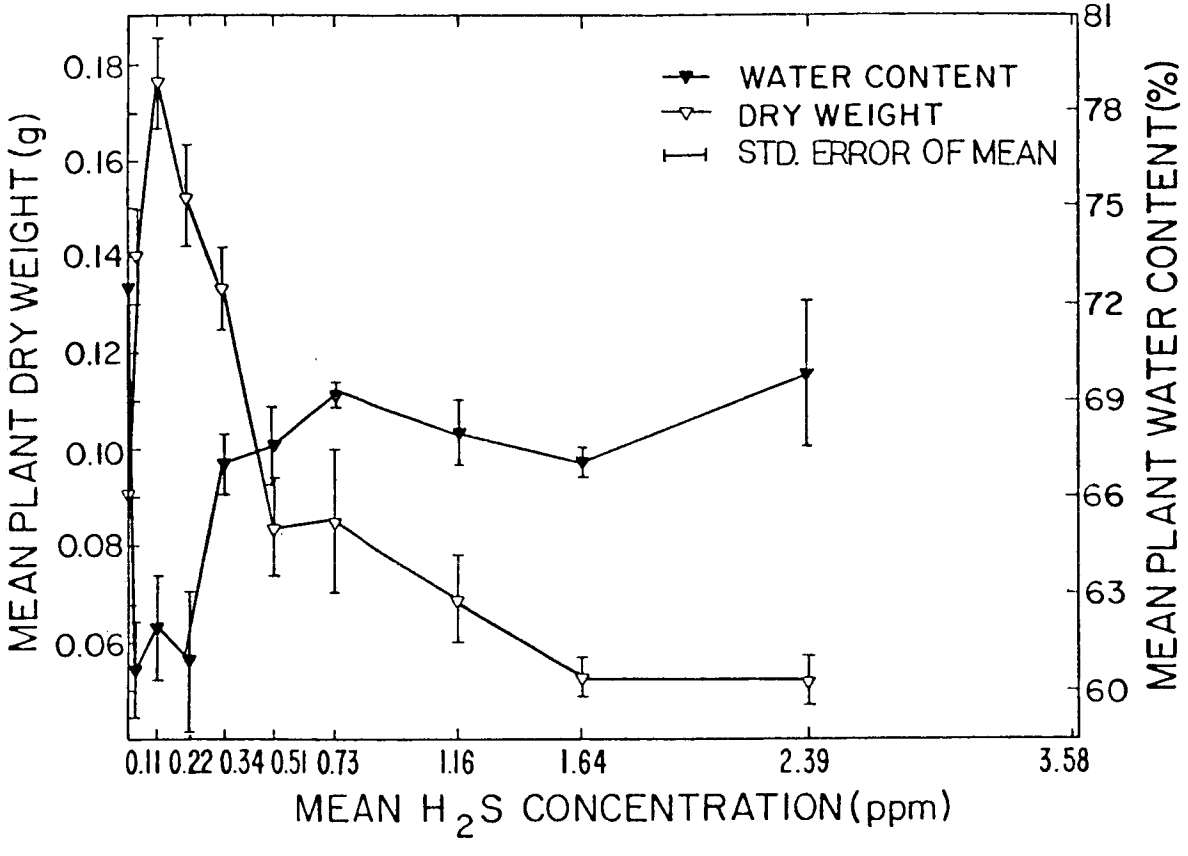

Fig. 9. Mean plant water content and plant dry weight of little bluestem topgrowth after fumigations with H_2S for 140 h over an 8-week period.

content of the plant may have actually enabled cells to function better because, as Salisbury and Ross (1978) reported, it is possible that cells function best when there is some water deficit. That is, there may be an optimum turgidity for cells above or below which the various plant functions are slower or less efficient (Salisbury and Ross, 1978). A stimulation of stomatal opening may have increased H_2S and CO_2 uptake to levels approaching optimum for utilization and growth. Thus, through the indirect mechanisms of increased CO_2 and H_2S uptake and utilization, and stimulation of plant cell function, the presence of low levels of H_2S may have been at least partially responsible for dry weight increases. At some concentration of H_2S, about 0.12 ppm, the stimulation effects may have been overcome by negative effects. At this "threshold" level plant dry weight began decreasing (Fig. 9). This may have been due to the plants' inability to continue "detoxifying" the H_2S through stress tolerance mechanisms. Figure 9 shows that as dry weight continues to decrease there is a corresponding increase in water content. As H_2S stress increased, there may have been a reduction in stomatal opening and/or a stimulation of stomatal closure which reduced transpiration and, therefore, water loss. Also, the lowering of plant water potential due to an ion increase would have increased plant water uptake. This process could have continued until water movement into cell protoplasts was no longer possible, thus causing the second decrease in water content.

Mountain Brome

The ANOVA table for water content of mountain brome topgrowth appears in Appendix Table 17. The F-test was barely significant indicating some difference existed between treatment means. Figure 8 shows that the water content of H_2S-treated plants increased and remained higher than that of the controls at all levels of H_2S. Multiple comparisons (Table 4) showed that only the mountain brome plants fumigated with the highest H_2S level (3.58 ppm) had a significantly higher water content mean than those which received no H_2S (controls). There was no significant difference between control plant means nor between means of H_2S-treated plants (Table 4). This indicated that the increase in water content of the H_2S-treated plants over that of the non-H_2S-treated plants may have been caused by some effect of the H_2S. The increase in water content of the H_2S-treated plants may have been due to partial stomatal closure and/or a water potential reduction.

Figure 10 shows the water content/dry weight relationship of the mountain brome plants. A correlation coefficient of 0.43 indicates a relatively moderate degree of relation between these response variables. A negative relationship is evident, further strengthing the results found with little bluestem.

Leaf Nitrogen Content

Little Bluestem

The ANOVA for mean leaf nitrogen content of little bluestem provided evidence that no real differences in treatment means existed (Appendix Table 18). Figure 11 shows that the mean leaf nitrogen content of the H_2S-treated plants was lower than that of the control plants at every level of H_2S; however, comparisons among treatment means (Table 5) showed that the only significant difference was between the control plant mean and the 0.05-ppm treatment mean. The mechanism by which H_2S reduced the nitrogen content of little bluestem leaves is unknown; however, this may be another indirect effect of H_2S-induced water stress. Steubing and Jäger (1978) reported a stimulation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzyme activity, a reduction in glutamic and aspartic acids, and an accumulation of free proline in pea (Pisum sativum L.) leaves fumigated with 2.1 ppm H_2S for 9 days. They attributed the proline accumulation to water stress conditions produced by the H_2S fumigations. Apparently, plants under water stress have the ability to synthesize large quantities of the amino acid proline via transfer of amino groups from glutamate and aspartate by transaminase enzymes such as GOT and GPT (Salisbury and Ross, 1978) The large quantity of proline which accumulates in the vacuoles would result in a highly negative osmotic potential in the protoplasts (Salisbury and Ross, 1979) possibly without the toxic effects of the H_2S ions,

Table 4. Comparisons among water content means of mountain brome plants using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11	12
Mean H_2S (ppm)	0	0	0	0	0.72	2.14	0.07	0.21	0.65	0.37	1.16	3.58
Mean Plant Water Content (%)	76.4	76.5	76.8	76.9	79.6	79.6	80.0	80.2	80.3	80.7	80.7	81.1

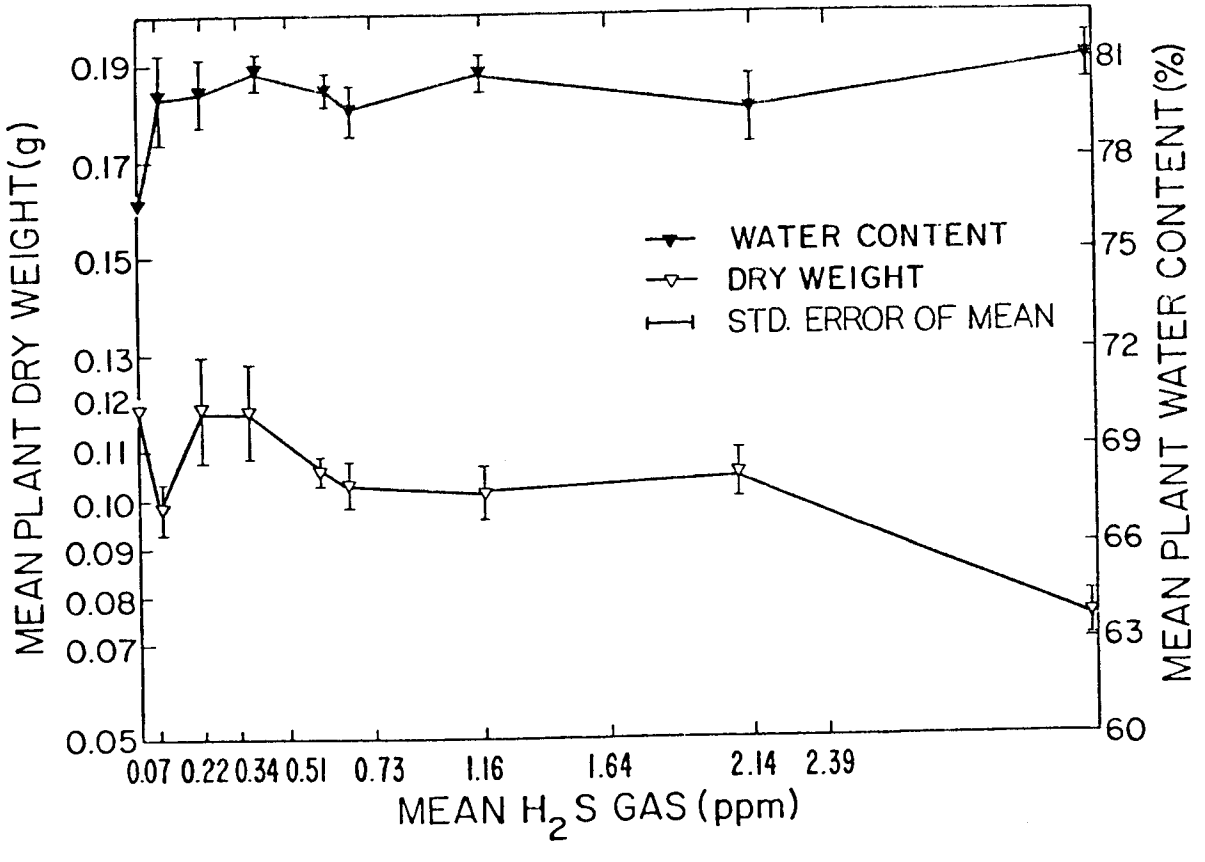

Fig. 10. Mean water content and dry weight of mountain brome topgrowth after fumigations with H_2S for 140 h over an 8-week period.

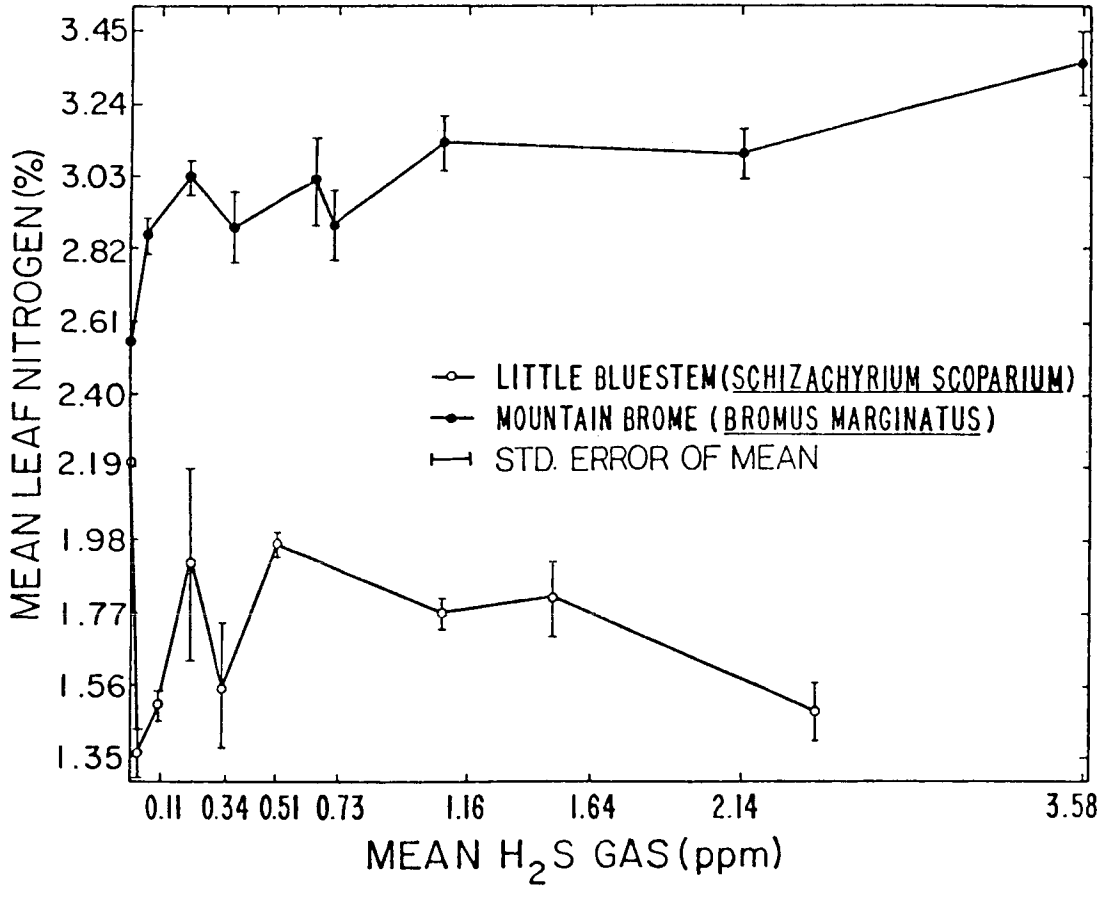

Fig. 11. Mean total-nitrogen content of mountain brome and little bluestem leaves after fumigations with H_2S for 140 h over an 8-week period.

Table 5. Comparisons among nitrogen content means of little bluestem leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9
Mean H_2S (ppm)	0.05	2.39	0.11	0.34	1.16	1.64	0.22	0.51	0
Mean Leaf Nitrogen (%)	1.37	1.49	1.53	1.55	1.77	1.81	1.92	1.97	2.20

but with the ability to allow water movement into the plant and protoplasts, thus hindering further water stress. This may explain the results reported by Steubing and Jäger (1978) and may possibly be related to the measured reductions in nitrogen in this study.

Figure 12 graphically displays the relationship between nitrogen content and water content along the H_2S gradient. The relatively moderate ($r = 0.59$) relationship between leaf nitrogen content and water content of topgrowth may possibly be attributed to the reasons discussed above.

Figure 13 depicts the relationship ($r = 0.16$) between leaf nitrogen content and dry weight of topgrowth of little bluestem. At the lower H_2S concentrations (0.05 to 0.12 ppm) dry weight increased to its highest level and nitrogen content decreased to its lowest level. Apparently the reduction in nitrogen content was not substantial enough to affect dry weight, or the stimulation effects of H_2S may have overcome the negative effects of the nitrogen content reductions, at least at the lower H_2S levels.

Mountain Brome

Figure 11 shows the mean leaf nitrogen content of mountain brome after the H_2S fumigations. A significant treatment effect was evident (Appendix Table 19). Table 6 shows that the grand mean leaf nitrogen content of the control plants was significantly lower than that of all the H_2S-treated means. Of the H_2S-treated means the only significant difference was between the 0.07- and the 3.58-ppm mean. This indicated that the fumigations with H_2S concentrations

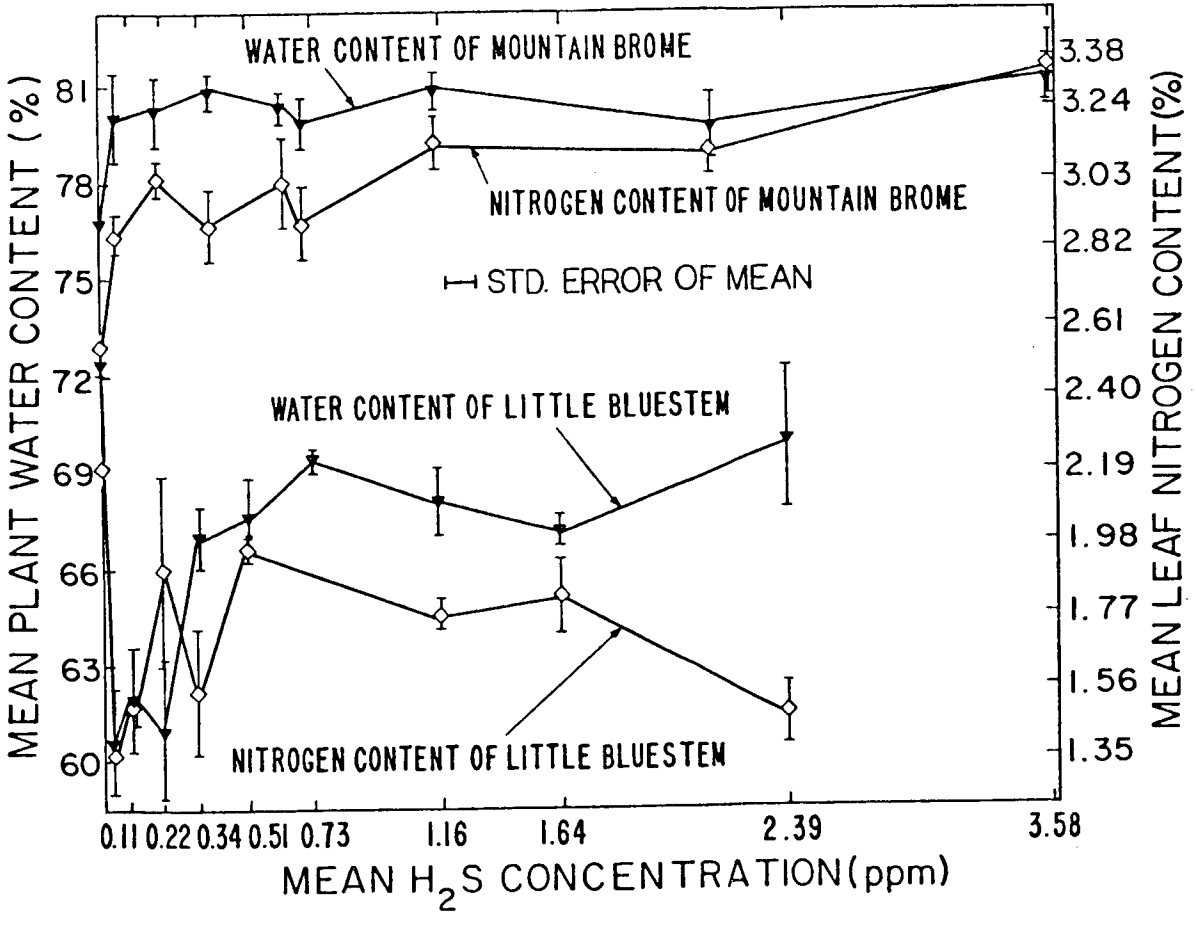

Fig. 12. Mean plant water content of topgrowth and leaf nitrogen content of little bluestem and mountain brome after fumigation with H_2S for 140 h over an 8-week period.

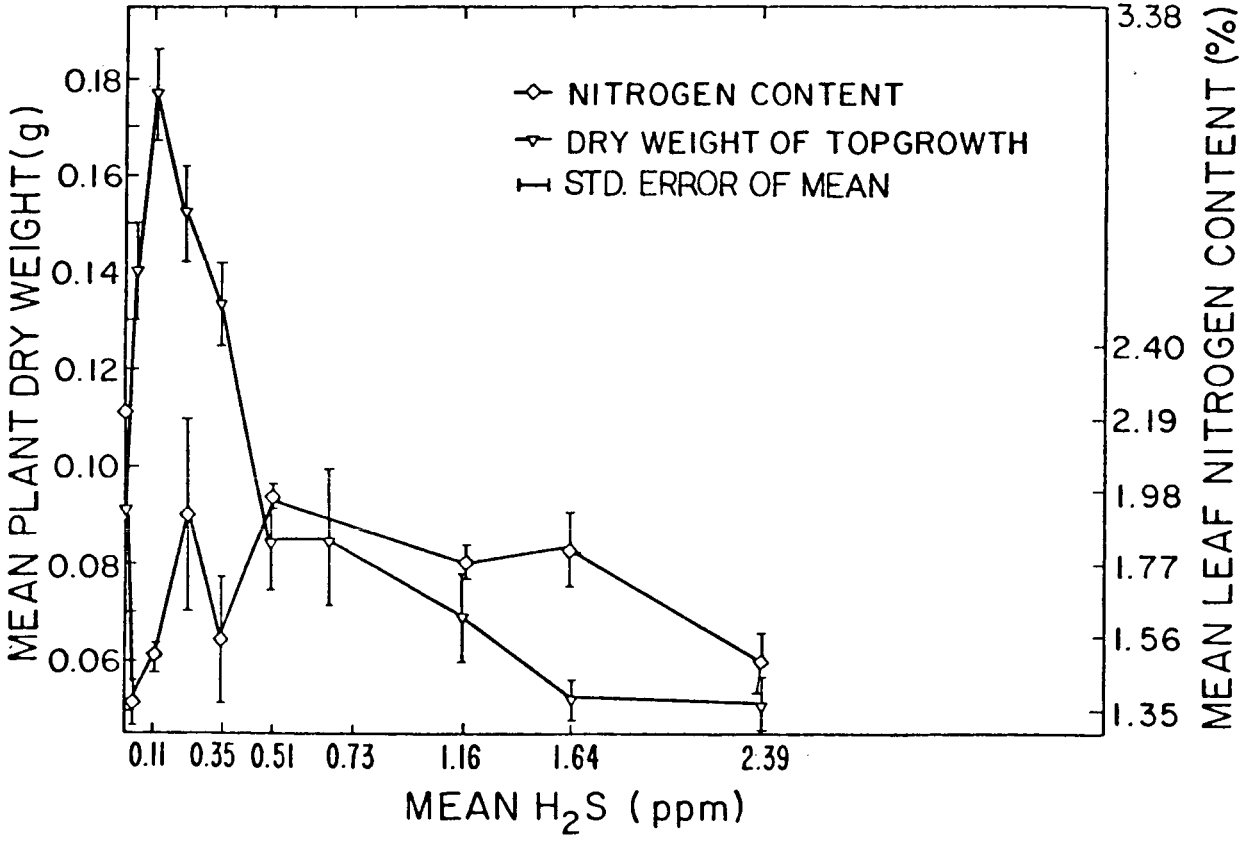

Fig. 13. Mean leaf nitrogen content and dry weight of topgrowth of little bluestem after fumigations with H_2S for 140 h.

Table 6. Comparisons among nitrogen content means of mountain brome leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11	12
Mean H_2S conc. (ppm)	0	0	0	0	0.07	0.37	0.72	0.65	0.21	2.14	1.16	3.58
Mean Leaf Nitrogen Content (%)	2.46	2.56	2.58	2.60	2.85	2.87	2.89	3.01	3.03	3.10	3.13	3.36

ranging from 0.07 to 2.14 ppm stimulated leaf nitrogen content to the same degree and the fumigations with 3.58 ppm H_2S resulted in a significantly higher mean leaf nitrogen content.

The responses of leaf nitrogen content and water content of topgrowth appear to have a strong ($r = 0.76$) positive relationship, as shown in Fig. 12.

Figure 14 depicts the relationship between leaf nitrogen content and dry weight of topgrowth of mountain brome plants fumigated with several levels of H_2S. The relationship ($r = 0.24$) was not strong. Thus, leaf nitrogen content and dry weight were not jointly affected by the external influences which included H_2S.

Leaf Chlorophyll Content

To facilitate discussion of the chlorophyll results, the length of time for which the groups of plants received the various fumigation treatments (exposure) will be identified as follows: 60 h = 4 h per day, 5 days per week for 3 weeks in succession; 80 h = 4 h per day, 5 days per week for 4 weeks in succession; 100 h = 4 h per day, 5 days per week for 5 weeks in succession; 120 h = 4 h per day, 5 days per week for 6 weeks in succession; 140 h = 4 h per day, 5 days per week over an 8-week period (there was a 9-day fumigation-free period between the 6th and 8th week after fumigations commenced).

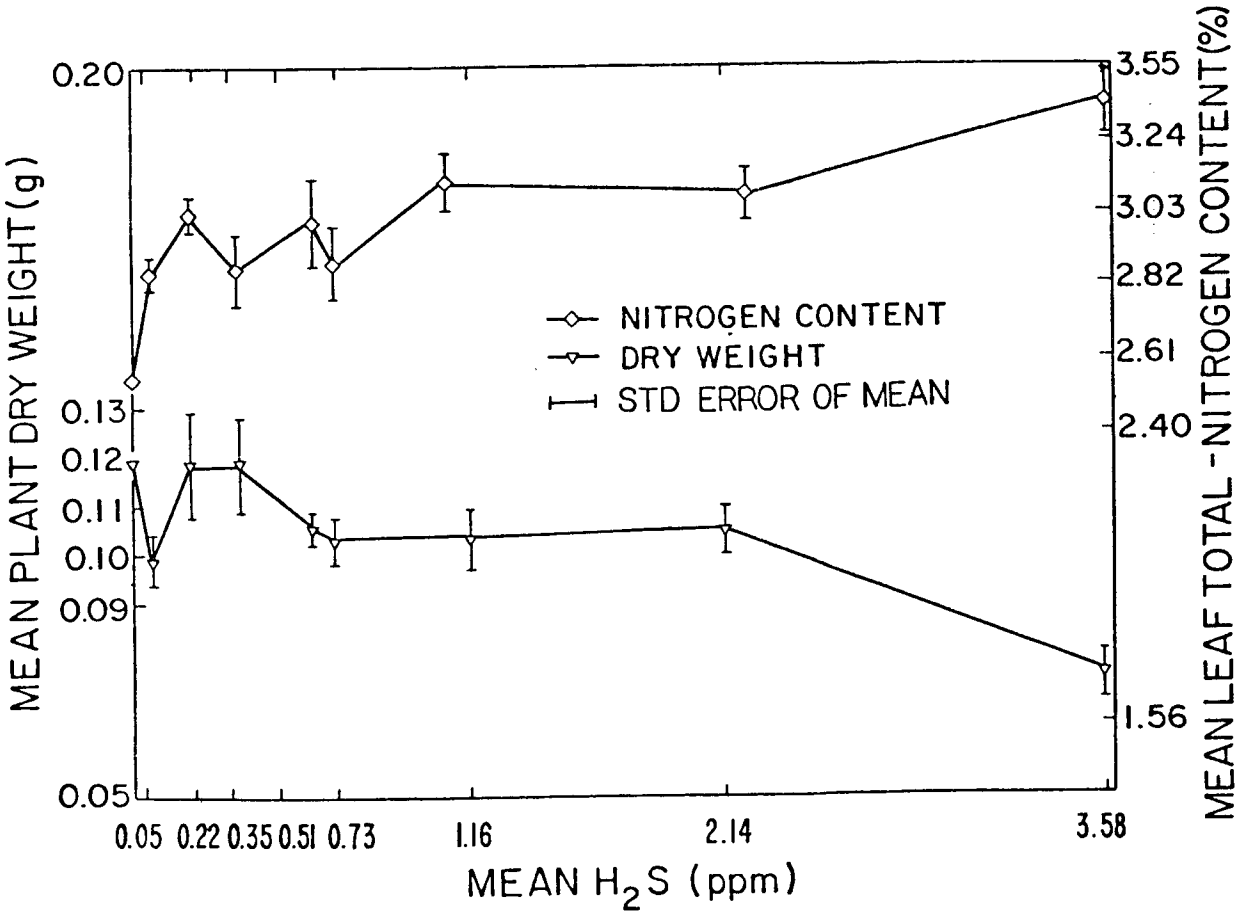

Fig. 14. Mean leaf nitrogen content and plant dry weight of topgrowth of mountain brome after fumigations with H_2S for 140 h.

Little Bluestem

Effects of exposure time. Chlorophyll content determinations of little bluestem leaf extracts were made after 60, 80, 100, and 140 total h of fumigation. The ANOVA tables for mean leaf chlorophyll content data appear in Appendix Tables 20 through 23. The leaf chlorophyll content at each of several mean H_2S concentrations was averaged across pots for each total-hour sampling time and appears in Fig. 15. Comparisons among treatment means for chlorophyll content data appear in Tables 7, 8, 9, and 10 for the 60-, 80-, 100-, and 140-h exposure times, respectively.

At the 60-h exposure time leaf chlorophyll content increased rapidly from the 0- to the 0.12-ppm level of H_2S, and then declined in general at a decreasing rate from the 0.12- to the 2.50-ppm level with a second peak being formed at 0.51 ppm (Fig. 15). The increase in chlorophyll content of the H_2S-treated plants above that of the control plants (no H_2S) can be interpreted as an H_2S-caused stimulation of chlorophyll synthesis. This stimulated chlorophyll production may have resulted partly from a greater rate of chloroplast division (Salisbury and Ross, 1978) which may have resulted from cells functioning better at some water deficit, as mentioned earlier. The treatment mean at the 0.12-ppm level of H_2S was significantly higher than that at all the other levels of H_2S; all other treatment means showed no significant differences (Table 7).

At the 80-h exposure time leaf chlorophyll content decreased rapidly from the 0- to the 0.11-ppm level of H_2S, and at a slower

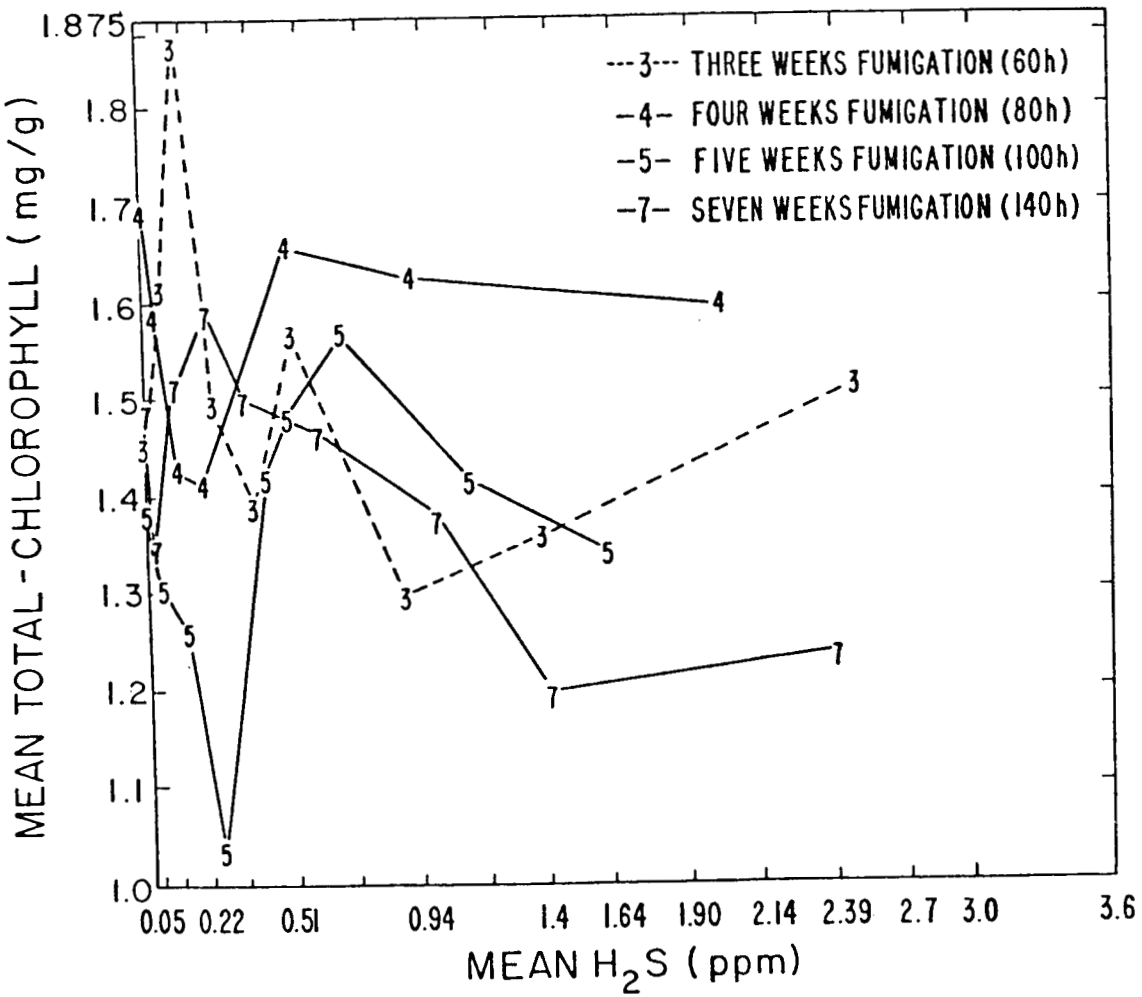

Fig. 15. Mean leaf chlorophyll content of little bluestem after 3,4,5 and 7 weeks of H_2S fumigations.

Table 7. Comparisons among chlorophyll content means of little bluestem leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 60 hours overs a three-week period. Any two means not underscored by the same line are significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9
Mean H_2S Conc. (ppm)	0.90	1.40	0.37	0	0.24	2.50	0.51	0.05	0.12
Mean Leaf Chlorophyll (mg/g)	1.295	1.352	1.388	1.452	1.489	1.513	1.566	1.599	1.863

Table 8. Comparisons among chlorophyll content means of little bluestem leaves using Duncan's Multiple-Range Test. Plants were fumigated with H_2S for a total of 80 hours over a four-week period

Rank	1	2	3	4	5	6	7
Mean H_2S Conc. (ppm)	0.21	0.11	2.03	0.05	0.93	0.45	0
Mean Leaf Chlorophyll (mg/g)	1.412	1.424	1.584	1.585	1.622	1.656	1.698

Table 9. Comparisons among chlorophyll-content means of little bluestem leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 100 hours over a five-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9
Mean H_2S (ppm)	0.26	0.12	0.06	1.62	0	1.10	0.38	0.47	0.67
Mean Leaf Chlorophyll (mg/g)	1.032	1.260	1.305	1.333	1.386	1.409	1.420	1.476	1.564

Table 10. Comparisons among chlorophyll content means of little bluestem leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over a seven-week period. Any two means underscored by the same line are not significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9
Mean H_2S Conc. (ppm)	1.41	2.39	0.04	1.01	0.58	0	0.33	0.10	0.22
Mean Leaf Chlorophyll (mg/g)	1.194	1.247	1.347	1.374	1.468	1.495	1.498	1.519	1.586

rate until 0.21 ppm had been received (Fig. 15). Leaf chlorophyll content then increased from the 0.21- to the 0.45-ppm level of H_2S forming a peak near that of the control, and then declined slowly from the 0.45- to the 2.03-ppm level. The control treatment mean was significantly higher than the 0.11- and the 0.21-ppm treatment means (Table 8).

At the 100-h exposure time leaf chlorophyll content followed a pattern similar to that of the 80-h exposure time (Fig. 15). There was a decrease in chlorophyll content from the 0- to the 0.26-ppm level of H_2S, and then an increase from the 0.26- to the 0.67-ppm level with a peak being formed at the 0.67-ppm level. Chlorophyll content then declined slowly again from the 0.67- to the 1.62-ppm level of H_2S. The 0.26-ppm treatment mean was significantly different than all the other treatment means (Table 9).

At the 140-h exposure time leaf chlorophyll content followed a pattern much like that of the 60-h exposure time without the second peak. There was an increase in chlorophyll content from the 0- to the 0.22-ppm level of H_2S, and then a gradual decrease from the 0- to the 2.96-ppm level. Table 10 shows that the 0.22-ppm treatment mean was significantly higher than the treatment means which received 1.41, and 2.39 ppm of H_2S. Also, the 1.41-ppm treatment mean was significantly lower than those which received 0, 0.10, 0.22, 0.33, and 0.58 ppm H_2S (Table 10).

The spectrophotometer absorbance measurements of the 140-h little bluestem leaf-chlorophyll extracts were made in triplicate in order to determine whether the variation contributed by the spectro-

photometer and its use was a statistically significant source of sampling error. The ANOVA table for these data appears in Appendix Table 23 and provides evidence that variation due to spectrophotometer sampling error contributed negligibly to the total error in determining leaf chlorophyll content.

Reductions in chlorophyll during a 4-h fumigation period. On a particular day midway through the 7th week of fumigations (140 h), leaf chlorophyll determinations were made on little bluestem extracts both before and after fumigations. A t-value of 0.51 indicated no significant differences among control-plant leaf chlorophyll content means before and after the fumigations at α=0.05. A t-value of 2.1 for the H_2S-treated plants indicated that there was a significant 7% decrease in mean leaf chlorophyll content following the fumigation at the 95% confidence level. The true mean difference in leaf chlorophyll content between the H_2S-treated plants before and after a 4-h fumigation lies between -0.066 and -0.108 mg/g of fresh weight or between 4.6 and 7.6% reductions.

Exposure interactions. Figure 16 represents the effect that the fumigations had on the leaf chlorophyll content of the H_2S-treated plants as the exposure time increased. At the lower H_2S fumigation levels (below 0.67 ppm) chlorophyll content decreased substantially from the 60- to the 100-h exposure time, and then increased from the 100- to the 140-h exposure time. This pattern can also be observed in Fig. 15. The increase in chlorophyll content from the 100- to the 140-h exposure time can be attributed to the 9-day fumigation-free period which the plants experienced between the

120- and the 140-h exposure time. The "recovery" period may have enabled the plants to resume a more normal rate of cell division and/or chlorophyll synthesis, resulting in leaf chlorophyll contents close to that measured at the 60-h exposure time. The dotted line in Fig. 16 is simply a linear extension of the 80- to the 100-h exposure time line which serves as a prediction of the change in chlorophyll content from the 100- to the 140-h exposure time if the H_2S-free period had not occurred.

At the higher H_2S fumigation levels (above 0.67 ppm) leaf chlorophyll content increased from the 60- to the 80-h exposure time, and then decreased from the 80- to the 140-h exposure time (Fig. 16). The absence of an increase in leaf chlorophyll content after the H_2S-free period indicates that the plants which received the higher levels of H_2S were damaged at the biochemical level to such a degree that they were unable to recover. Taylor (1978) defined this as prolonged elastic strain culminating in irreversible plastic strain.

Air temperature interaction. In order to determine whether the change in chlorophyll content over time was affected by environmental variables average weekly temperature over the 4 h daily fumigation period was used to represent changes in ambient environmental variables. Average air temperature of the week that chlorophyll determinations were made was plotted against time and compared with plots of chlorophyll content against time (Fig. 17). Air temperature was used as the representative variable because it may be the ambient environmental factor with the most significant

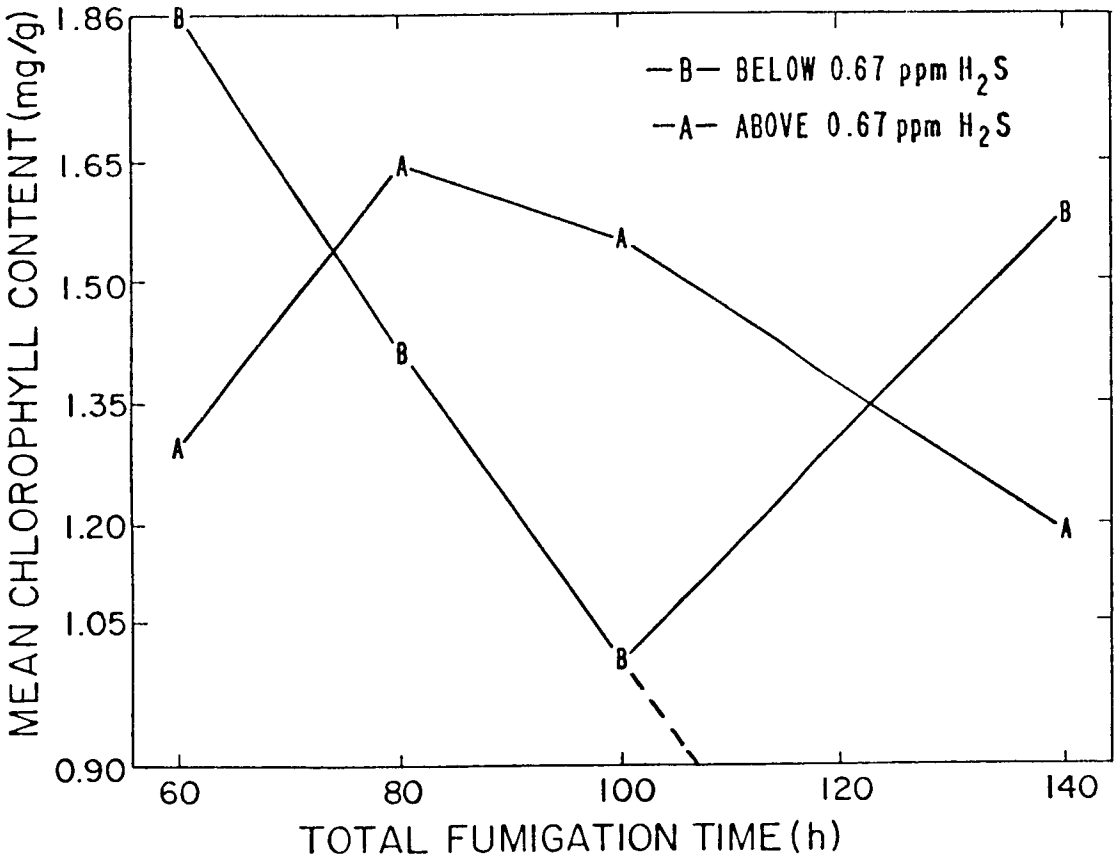

Fig. 16. Change in leaf chlorophyll content of little bluestem as total H_2S fumigation time increased.

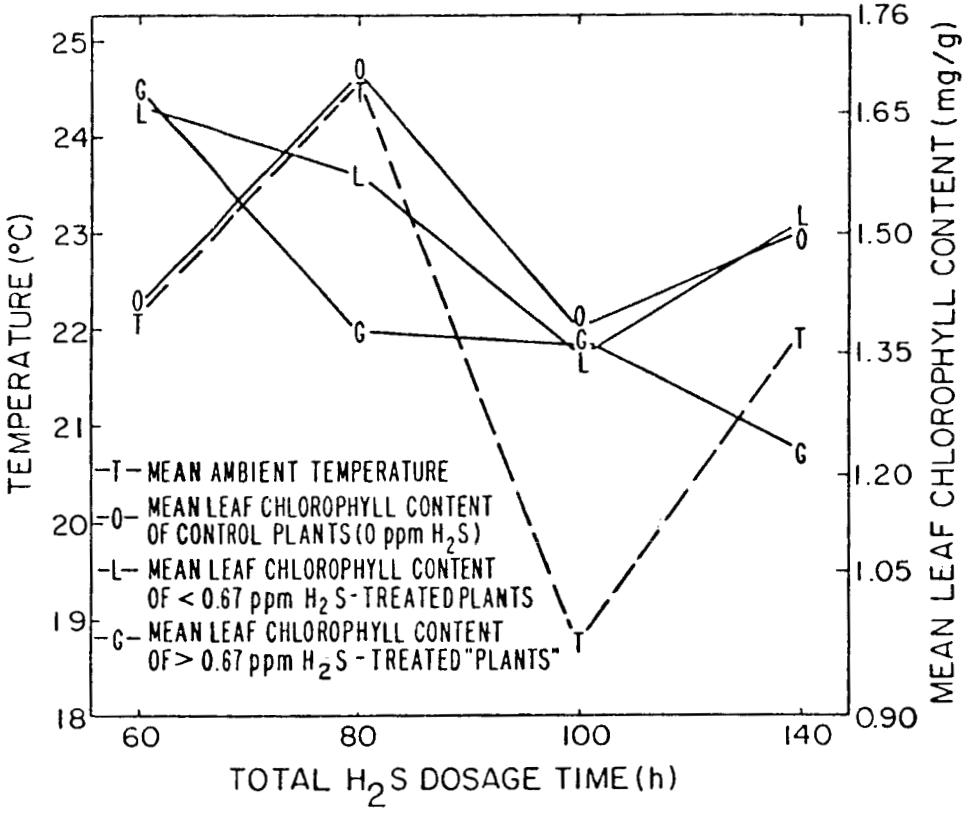

Fig. 17. Change in ambient air temperature and leaf chlorophyll content of little bluestem as total H_2S fumigation time increased.

influence on air pollutant effects on plants (Heck et al., 1965; Heck, 1968; Omrod, 1978) and it represents change in other ambient environmental variables including irradiance and humidity. Figure 17 shows that the change in control-plant leaf chlorophyll content over time followed the change in mean weekly ambient temperature quite consistently. A correlation coefficient of 0.69 indicates that this relationship is moderately strong. The relationship between chlorophyll content of the H_2S-treated plants and temperature is moderate as indicated by correlation coefficients of 0.45 and 0.39 for the low (< 0.67 ppm) and high (> 0.67 ppm) H_2S-level curves. Thus, leaf chlorophyll content of the control plants was affected to a greater degree by ambient temperature than that of H_2S-treated plants. This implies that temperature may have accounted for more of the variation in leaf chlorophyll content of plants that received no H_2S than in H_2S-treated plants. However, the amount of variation in chlorophyll content contributed by air temperature is unknown.

Plant response relationships. The relationship between leaf chlorophyll content and the other response variables of water content, nitrogen content, and dry weight are shown separately in Figs. 18, 19, and 20, respectively. The relationship between chlorophyll content and water content in response to the fumigations was a negative one; water content increased as chlorophyll content decreased along an increasing level of H_2S (Fig. 18). This supports and may be explained by the statement alluded to earlier

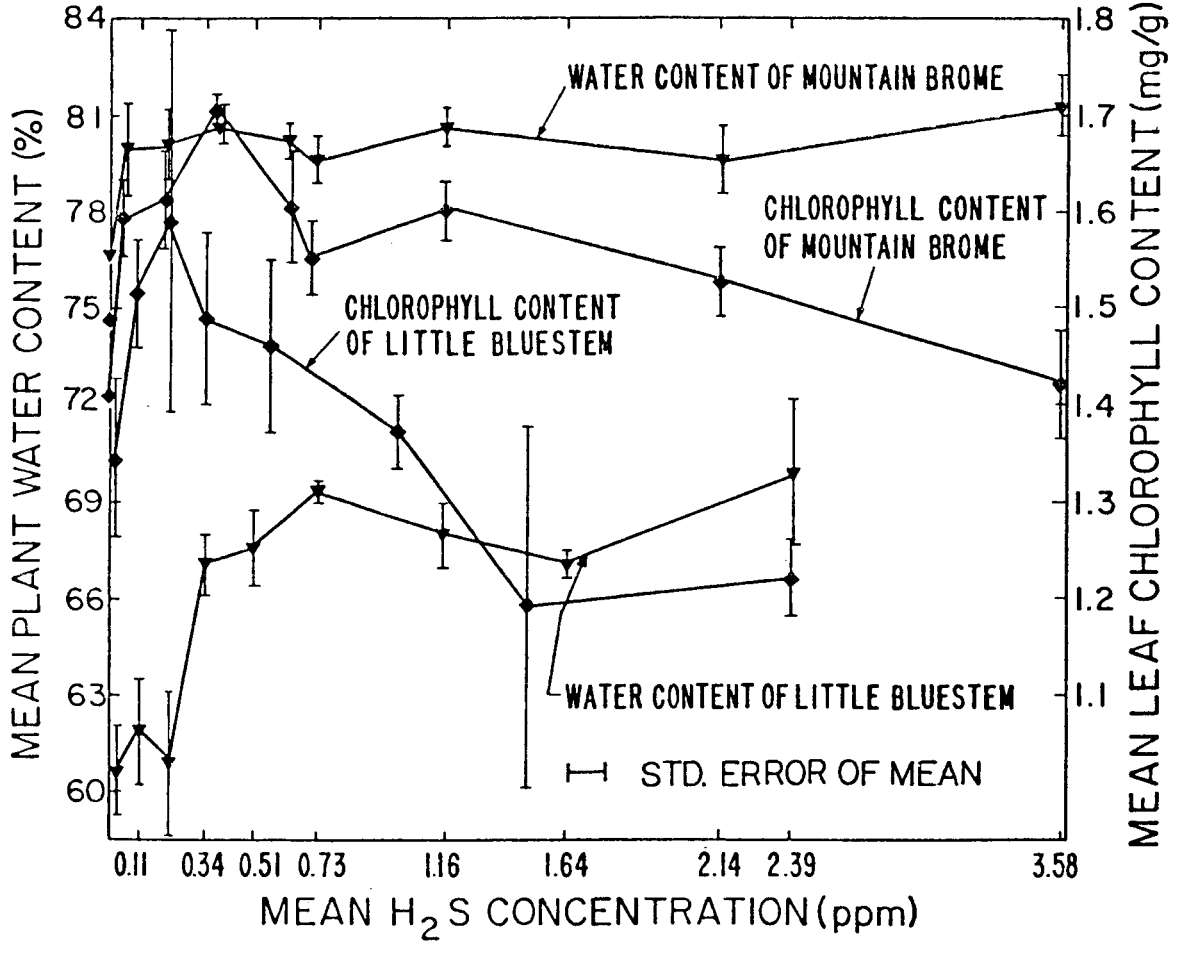

Fig. 18. Mean water content of topgrowth and leaf chlorophyll content of little bluestem and mountain brome fumigated with H_2S for 140 h over an 8 week period.

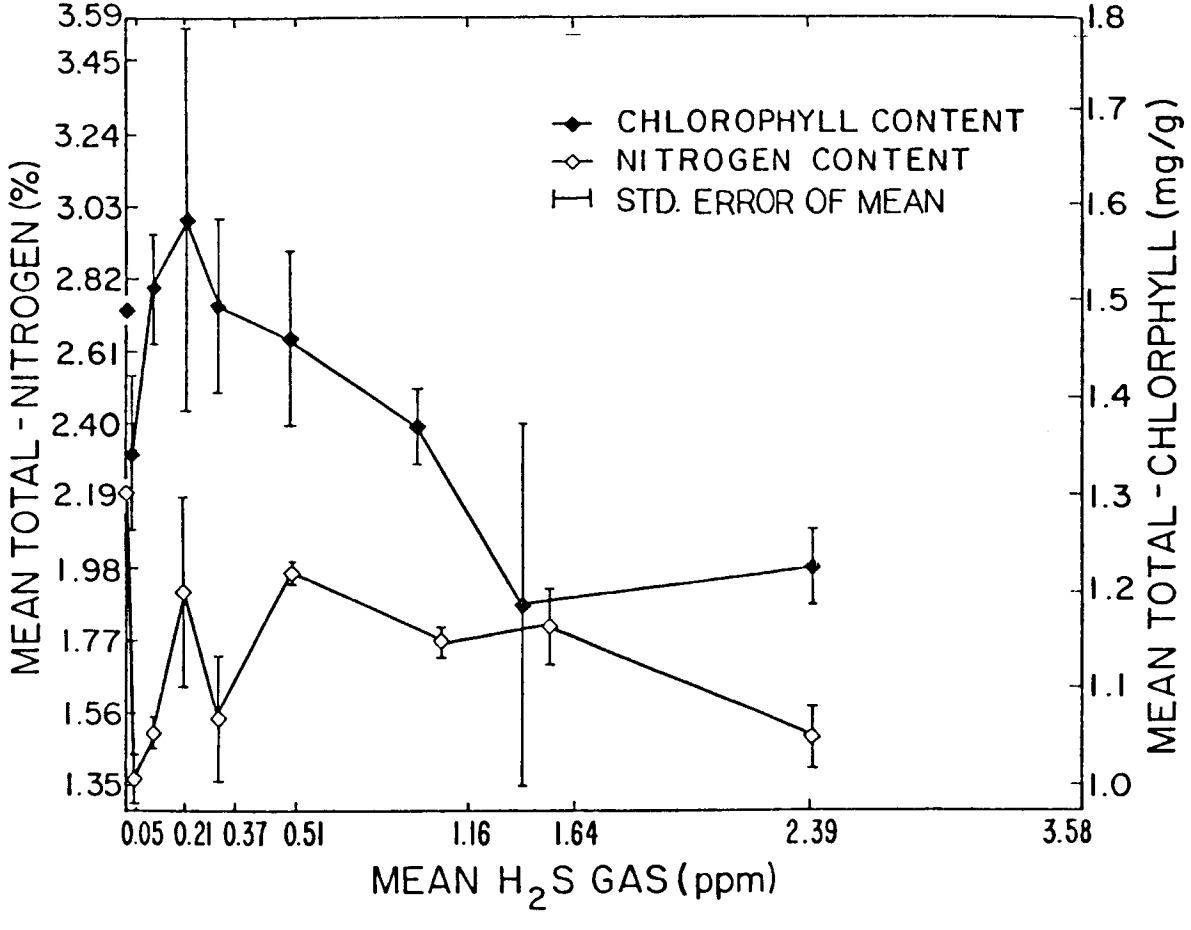

Fig. 19. Mean total-nitrogen and total-chlorophyll content of little bluestem leaves fumigated with a range of H_2S gas (in ppm).

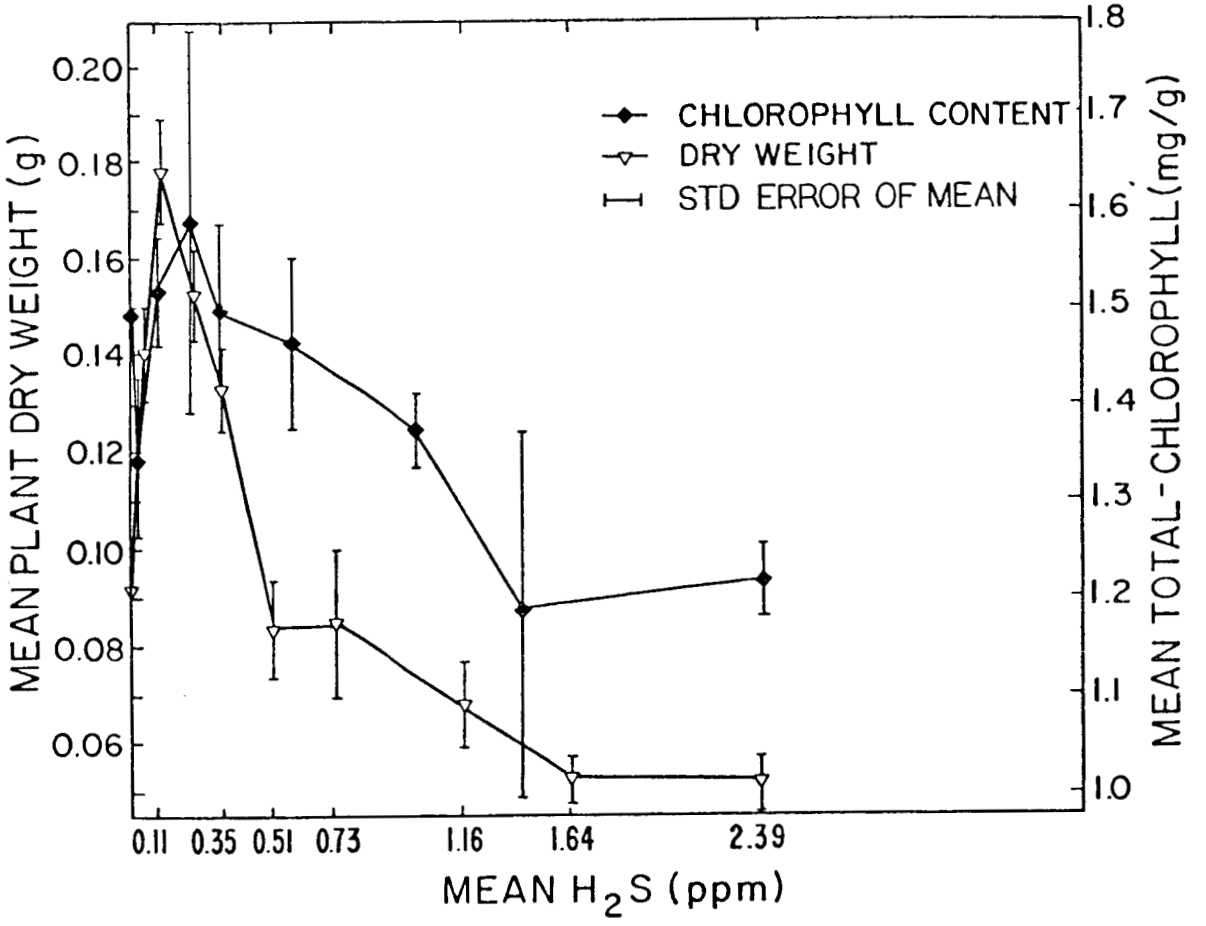

Fig. 20. Mean leaf chlorophyll content and dry weight of topgrowth of little bluestem plants fumigated with a range of H_2S for 140 h.

that cells function faster or more efficiently when there is some water deficit.

The positive relationship between chlorophyll and nitrogen in their responses to the H_2S fumigations (Fig. 19) may be expected since nitrogen is a major component of pyrole rings in chlorophyll (Salisbury and Ross, 1978). Both leaf chlorophyll and leaf nitrogen content decreased from the 0- to the 0.07-ppm level of H_2S, increased from the 0.07- to the 0.22-ppm level, and then decreased again from the 0.22- to the 0.33-ppm level. Above the 0.33-ppm level of H_2S, factors other than nitrogen content may have contributed more to the decline of leaf chlorophyll content than at the levels below 0.33 ppm.

A strong ($r = 0.60$) positive relationship between leaf chlorophyll content and dry weight is evident in Fig. 20. An increase in chlorophyll content could have led to an increase in light trapping and therefore, a stimulation of photosynthesis resulting in greater dry weight production. Above the 0.33-ppm level of H_2S, it appears that leaf chlorophyll content contributed less to the change in dry weight than below the 0.33-ppm level (Fig. 20) as was the case with leaf nitrogen content. Thus, most of the correlation can be attributed to the lower end of the relationship.

Mountain Brome

Effects of exposure time. Leaf chlorophyll content determinations of mountain brome were made after 60, 80, 120, and 140 total h of fumigation. The ANOVA tables for mean leaf chlorophyll content

data appear in Appendix Tables 24 through 27. The chlorophyll content at each of several mean H_2S concentrations was averaged across pots for each subplot and appears in Fig. 21. Comparisons among treatment means for chlorophyll content data appear in Tables 11 and 12 for the 120- and the 140-h exposure times, respectively.

At the 60-h exposure time leaf chlorophyll content remained relatively unchanged (Fig. 21). No treatment differences were detected (Appendix Table 24).

At the 80-h exposure time there was an extremely small, insignificant change in leaf chlorophyll content across all levels of H_2S (Fig. 21). Again, no treatment differences were evident (Appendix Table 25).

At the 120-h exposure time the F-value for testing treatment differences was barely significant at the 95% confidence level. There was a general decrease in leaf chlorophyll content from the 0- to the 2.14-ppm level of H_2S, and then an increase from the 2.14- to the 3.58-ppm level. The 2.14-ppm treatment mean differed significantly from the control-plant grand mean and from the 0.21-ppm treatment mean (Table 11).

At the 140-h exposure time leaf chlorophyll content increased significantly from the 0- to the 0.37-ppm level of H_2S, and then decreased significantly from the 0.37- to the 3.58-ppm level (Fig. 21 and Table 12). There were no significant differences between the control treatment means indicating no significant variation in leaf chlorophyll content from chamber to chamber.

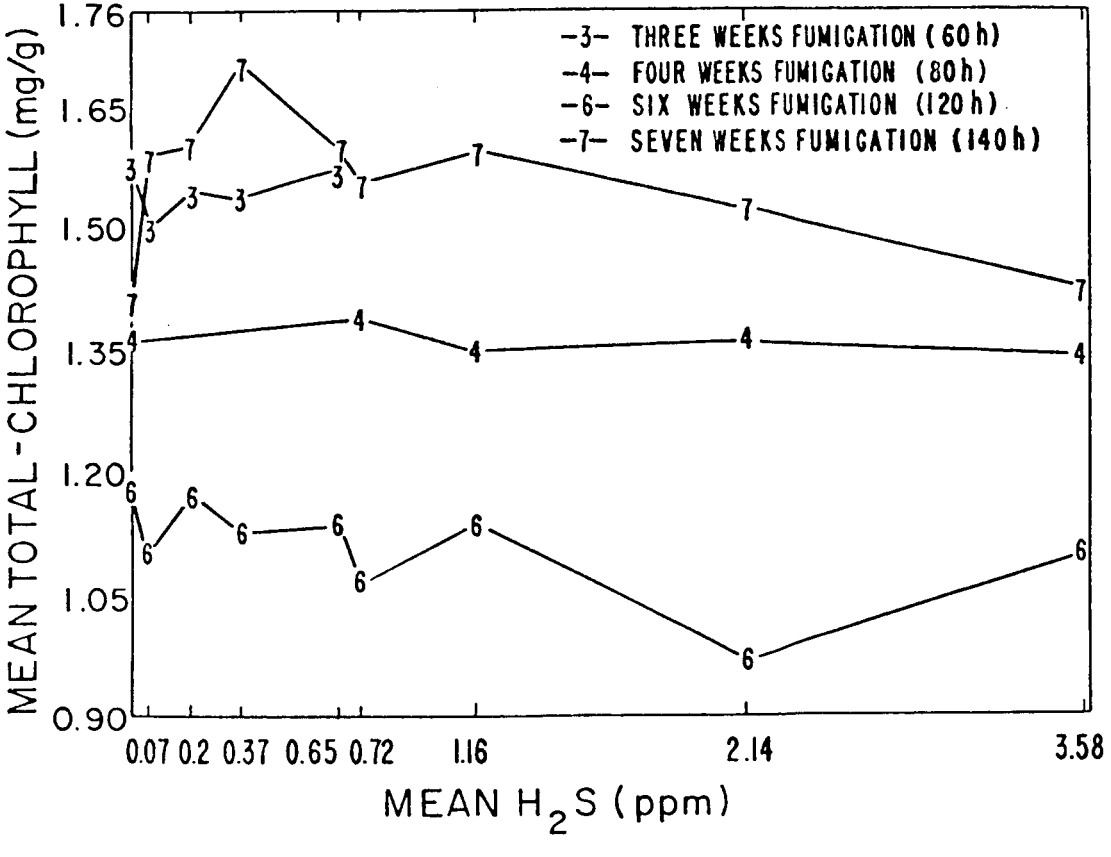

Fig. 21. Mean leaf chlorophyll content of mountain brome after 3,4,6 and 7 weeks of H_2S fumigations.

Table 11. Comparisons among chlorophyll content means of mountain brome leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 120 hours over a six-week period. Any two means not underscored by the same line are significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11	12
Average H_2S Conc. (ppm)	2.14	0.72	3.58	0.07	0	0.37	0	0.65	1.16	0	0.21	0
Mean Leaf Chlorophyll (mg/g)	0.97	1.07	1.09	1.09	1.12	1.13	1.14	1.14	1.14	1.17	1.18	1.30

Table 12. Comparisons among chlorophyll content means of mountain brome leaves using Duncan's New Multiple-Range Test. Plants were fumigated with H_2S for a total of 140 hours over an eight-week period. Any two means not underscored by the same line are significantly different at the 95% confidence level.

Rank	1	2	3	4	5	6	7	8	9	10	11
Mean H_2S conc. (ppm)	0	0	0	3.58	2.14	0.72	0.07	1.16	0.65	0.21	0.37
Mean Leaf Chlorophyll (mg/g)	1.39	1.41	1.42	1.43	1.53	1.55	1.59	1.60	1.60	1.61	1.70

Exposure interactions. As was the case with little bluestem, it was evident that exposure time may have affected the leaf chlorophyll content of the H_2S-treated mountain brome plants (Fig. 21). Figure 22 represents the change in leaf chlorophyll content of mountain brome as exposure time increased. Chlorophyll content decreased substantially from the 60- to the 120-h exposure time, and then increasesd from the 120- to the 140-h time to a level above that at the 60-h time. This pattern can also be observed in Fig. 21. The increase in chlorophyll content from the 120- to the 140-h exposure time (Fig. 22) was again attributed to the 9-d H_2S-free period which the mountain brome plants experienced between the sixth and seventh week after fumigations were commenced. The "recovery" period may have enabled the plants to resume a rate of chlorophyll synthesis that resulted in a mean chlorophyll content above that of the 60-h exposure time. The dotted line in Fig. 22 is a linearly extended prediction of the change in chlorophyll content from the 120- to the 140-h time if the "recovery" period had not occurred.

Air temperature interaction. The change in ambient air temperature as exposure time increased is compared with the change in leaf chlorophyll content of mountain brome as exposure time increased in Fig. 23. A relatively strong ($r = 0.70$) negative relationship between leaf chlorophyll content and ambient air temperature is evident. As the exposure time increased from 60 to 120 h, air temperature increased and chlorophyll content decreased for both the control and the H_2S-treated plants. Then the air temperature decreased substantially and the chlorophyll contents increased from

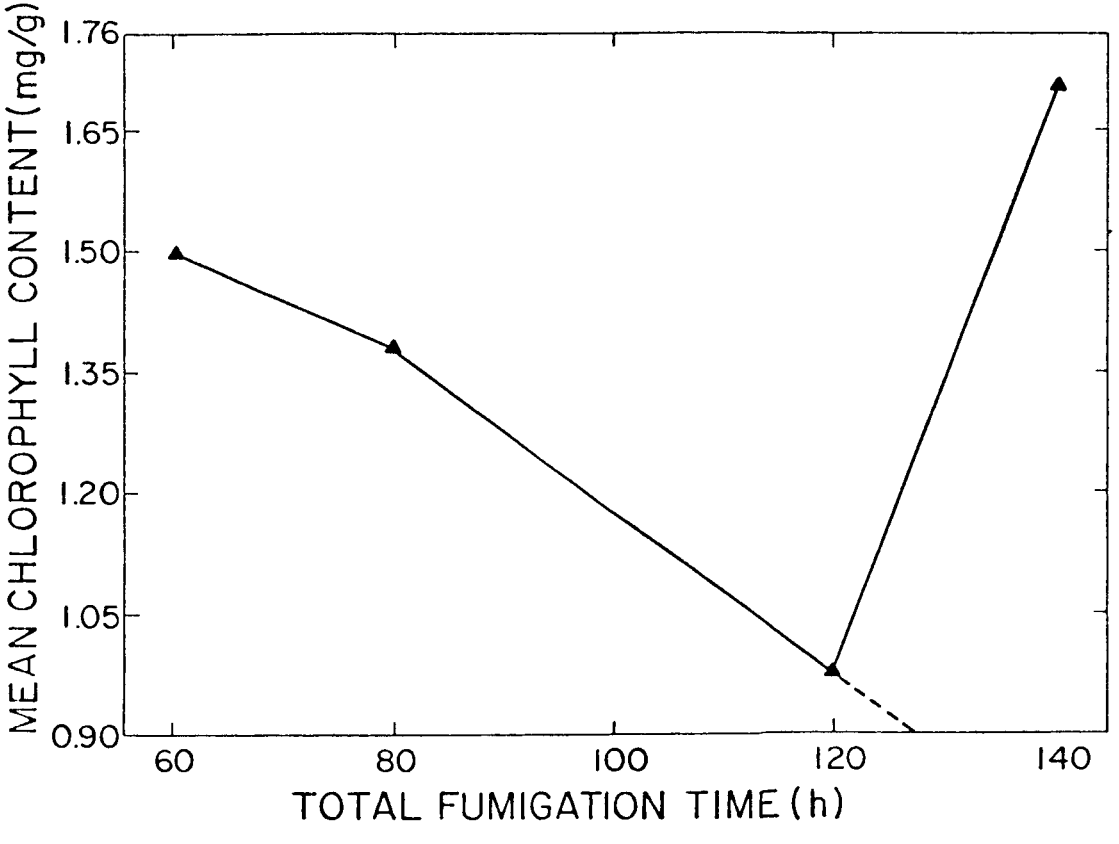

Fig. 22. Changes in leaf chlorophyll content of mountain brome as total fumigation time increased.

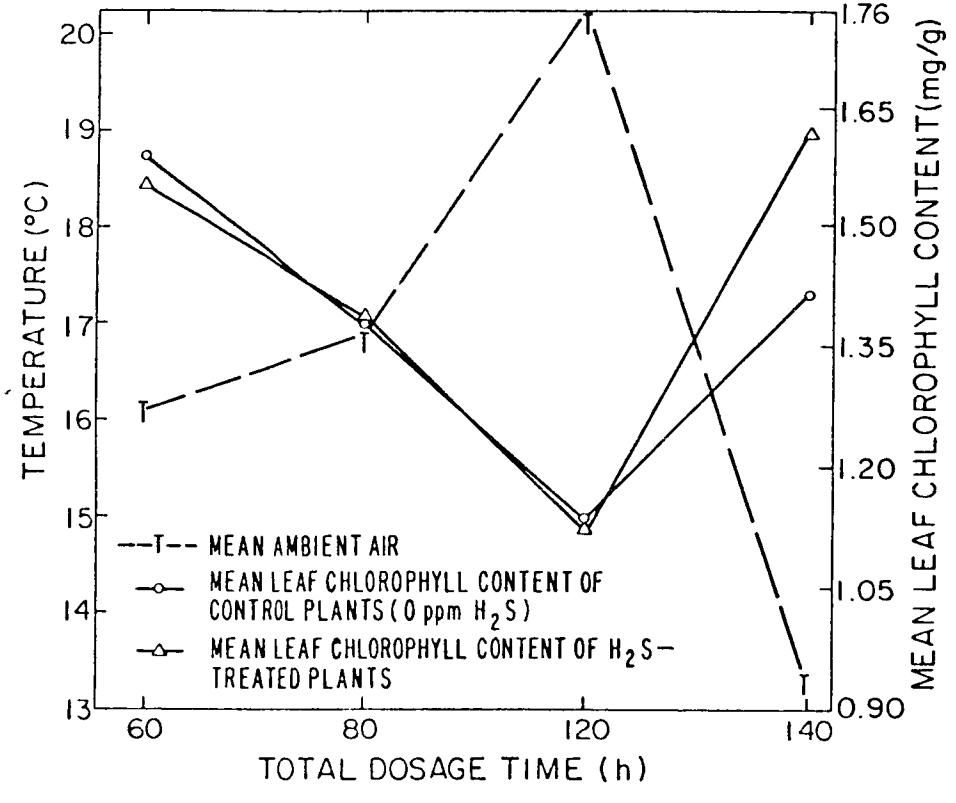

Fig. 23. Changes in ambient air temperature and leaf chlorophyll content of mountain brome as total H_2S fumigation time increased.

the 120- to the 140-h exposure time. The closeness in pattern between the control and the H_2S-treated plants indicated that they were affected similarly by the extraneous variables, which included air temperature. The strong negative relationship between changes in leaf chlorophyll content and ambient air temperature has two implications. Firstly, the strength of the relationship implies that temperature contributed substantially to the changes in leaf chlorophyll content over time. The amount of variation in leaf chlorophyll content contributed by air temperature can only be determined by partitioning out this contribution using analyses of variance with multiway classifications or factorial experiments. This requires sampling at the same H_2S level at each sampling date, a procedure which was not done in this experiment. Secondly, the negative aspect of the relationship implies that mountain brome may be adapted to an altitude of colder air temperatures than the altitude at which the experiments were conducted or to growth during a colder time of season. This deduction is based on the fact that the maximum measured chlorophyll content was reached at the coldest air temperature (Fig. 23). Chase (1979) reported that mountain brome is common to the latitude and longitude of New Mexico, therefore, only a change in altitude or growing season would satisfy the colder air temperature requirement.

Plant response relationships. The relationship between leaf chlorophyll content and the other response variables, of water content of topgrowth, leaf nitrogen content, and dry weight of topgrowth, are shown separately in Figs. 18, 24, and 25, respectively.

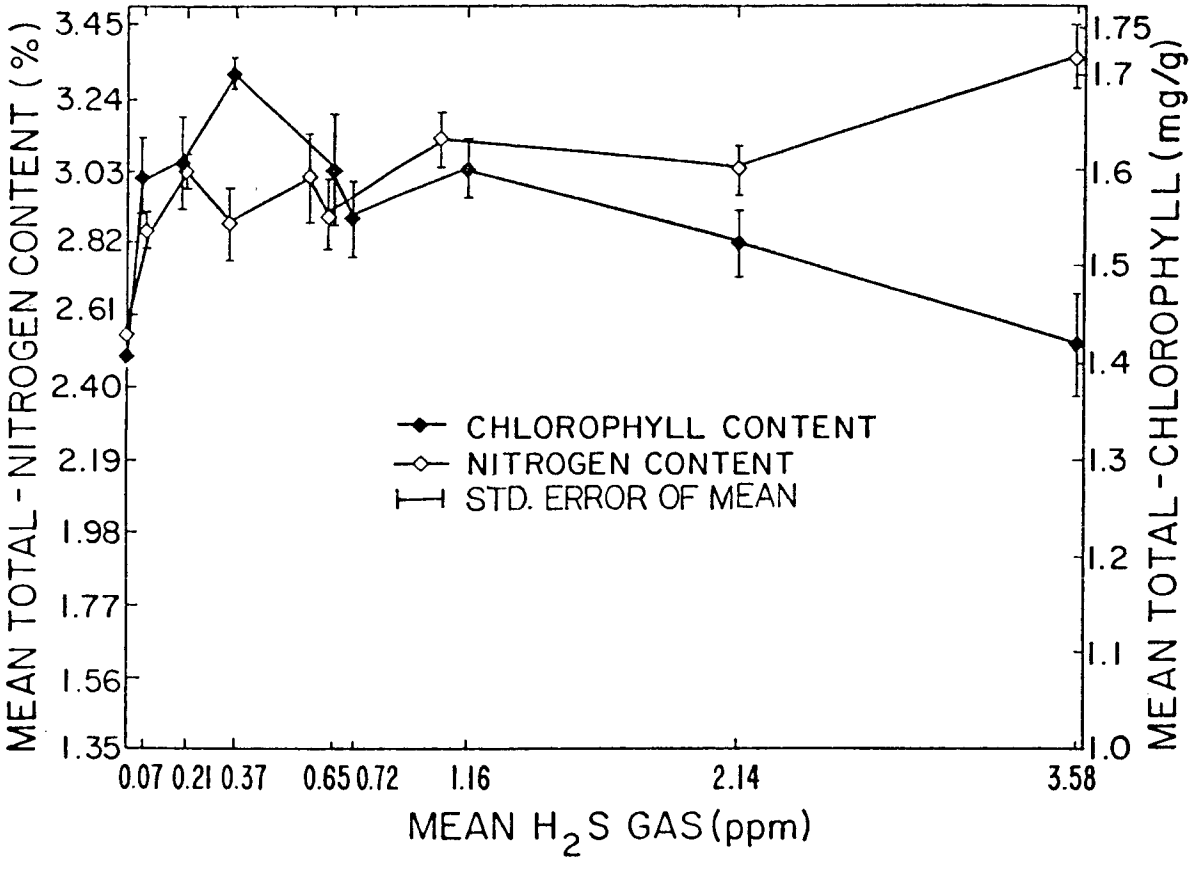

Fig. 24. Mean total-nitrogen and total-chlorophyll content of mountain brome leaves fumigated 140 h with H_2S.

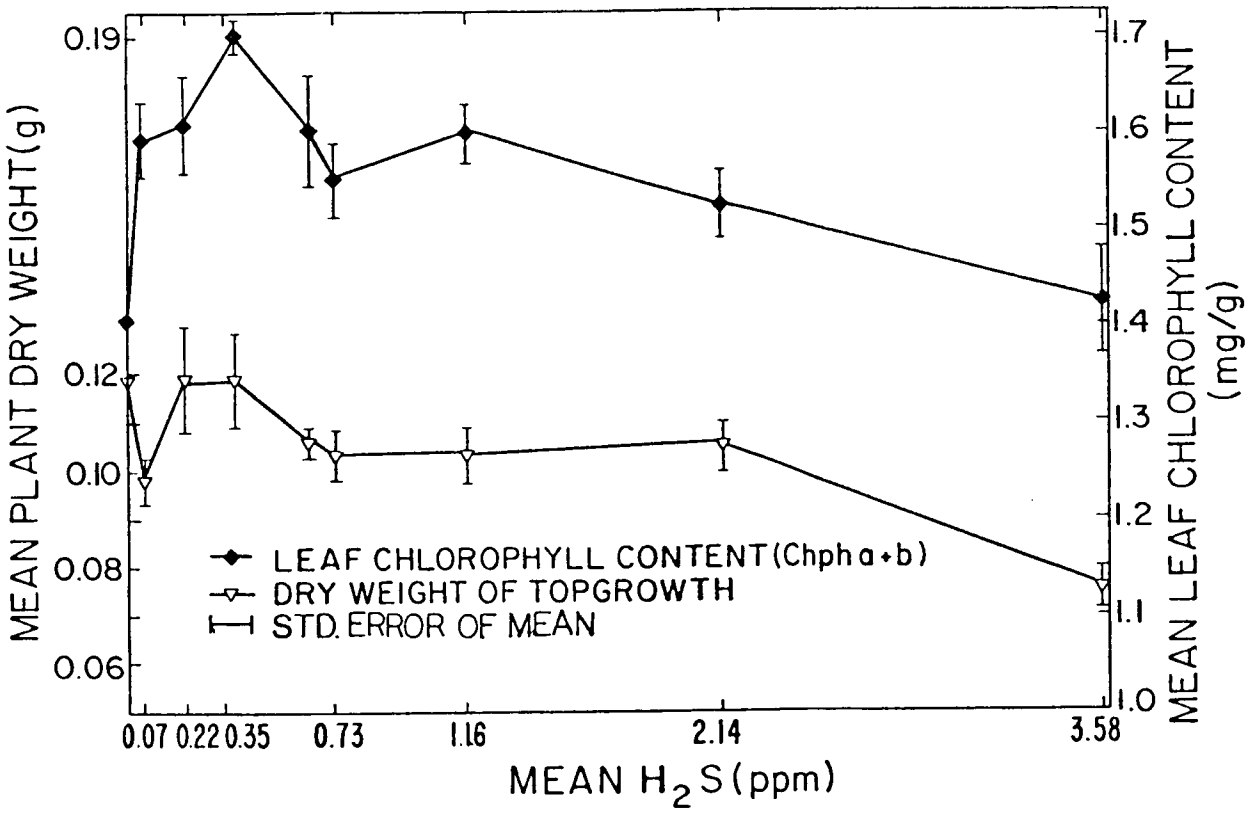

Fig. 25. Mean plant dry weight of topgrowth and leaf chlorophyll content of mountain brome fumigated with H_2S for 140 h.

A positive ($r = 0.50$) relationship between chlorophyll content and water content along an increasing H_2S gradient is shown in Fig. 18. This encourages the "better cell functioning at some water deficit" theory. Generally, as water content increased from the 0- to 0.37-ppm level of H_2S so did chlorophyll content, and then chlorophyll content decreased by about 16% from the 0.37- to the 3.58-level of H_2S as water content remained about the same. Thus, above the 0.37-ppm level of H_2S, chlorophyll and water content may have ceased to respond similarly to the H_2S fumigations.

A positive ($r = 0.32$) relationship between chlorophyll and nitrogen content along an increasing H_2S gradient is shown in Fig. 24. The relationship is not strong and occasionally turns to a negative relationship.

A positive ($r = 0.42$) relationship between dry weight and chlorophyll content along an increasing H_2S gradient is plotted in Fig. 25. However, the loss in dry weight ($\sim$37%) over the entire spectrum of H_2S dosages is not proportional to the change in chlorophyll content, which, in fact, was an increase of about 2%.

Figure 26 represents the change in leaf chlorophyll content of little bluestem and mountain brome as H_2S concentration increased.

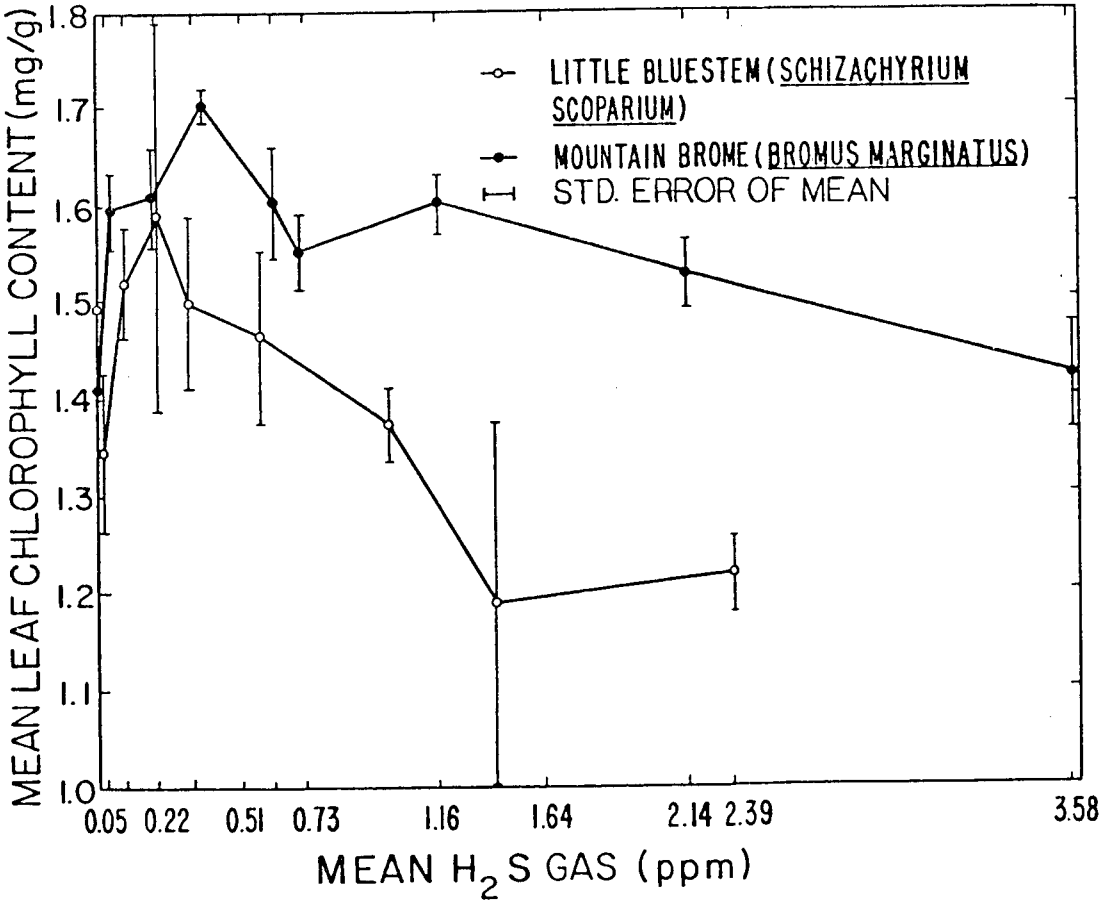

Fig. 26. Mean total-chlorophyll content of mountain brome and little bluestem leaves fumigated with H_2S for 140 h over an 8-week period.

DISCUSSION

After reviewing the literature it seems evident that after H_2S is released into the troposphere much of it will be exposed to plants in an unaltered form. The long residence time of H_2S before it is converted to SO_2 supports this statement. Hydrogen sulfide as a gas is about 16% heavier than air (Hendrickson, 1979); therefore, it seems possible that some settling may occur exposing some vegetation to slightly higher concentrations of H_2S than that found above a canopy layer. However, the atomic weight of CO_2 is greater than that of H_2S. Thus, settling of geothermal gases must be considered since CO_2 may ameliorate the effects of H_2S on plants (Shinn et al., 1977). Special problems may develop in mountainous regions where air drainage patterns result in gas build-ups in valleys and canyons. Since it is likely that undissociated H_2S crosses biologic membranes more rapidly than the charged anionic species (Smith, 1979), and H_2S is the form common to the troposphere, it seems feasible that there would be less stress avoidance and more stress tolerance, as described by Taylor (1978), in plants exposed to H_2S.

Plant species express wide variations in their responses to gaseous air contaminants (Mudd, 1975; Bradshaw, 1976; Smith, 1979). This variability is exhibited both between species and within species. However, there is evidence of populations showing specific adaptations or responses to most known pollutants (Bradshaw, 1976).

After having reviewed the literature on H_2S effects on plants and considering the results of this study, a generalized pattern of plant reponses to H_2S can be developed. This is shown in Table 13. It is important to note that the latter events are most likely indirect responses to one or more of the earlier events rather than to H_2S stress per se.

Initially most of the absorbed sulfur is incorporated into organic compounds (Steubing and Jäger, 1978). There is a stimulation of many growth processes and, if gas concentrations are non-acute, there is a stimulation of growth. Those amounts of sulfur which are taken up in excess are accumulated in the vacuole as sulfate, which causes an increase in osmotic pressure of the cell sap (Steubing and Jäger, 1978). The osmotic potential of the cell protoplasts continue to become more negative due to storage of HS^- and $SO_4^=$ ions in the vacuole. Water continues to enter the protoplast because of the universal tendency to establish equilibrium in water potentials between the apoplast and the protoplast, thus creating positive protoplastic pressure potentials. This continues until the protoplast can no longer accept anions or water due to a high water potential caused by the osmotic pressure or due to the fact that the protoplast has reached its stretching capacity. The excess sulfate which can no longer be stored in the vacuoles lowers the water potential in the apoplast below that in the protoplast. As a result, water moves out of the protoplasts of mesophyll and epidermal cells and plants become water stressed (Meidner and Sheriff, 1976). This may explain the water-stress conditions

Table 13. Generalized pattern of plant processes or responses to increasing hydrogen sulfide levels under optimal or near-optimal environmental conditions.[a]

Process or Response Affected[c]	H_2S Dosage[b] Concentration x Length of Exposure				Remarks
	0 ppm h	150 ppm h	400 ppm h	650 ppm h	
Stimulation of growth processes					
Protoplastic Ψ_m ↑ and Ψ_p ↑	———	– –			
Cell growth ↑	———	– – – –			
Wall synthesis ↑	———	– –			
Protein synthesis ↑	———	– –			
Stomatal opening ↑	– – – –	———	– – – – – –		Depends on Species
CO_2 assimilation ↑	– – – –	———	– – – – – –		Depends on Species
Overall plant growth ↑	– –	———	– – – – – – –		
Suppression of growth processes					
Protoplastic Ψ_p ↓ and Ψ_m ↔		– –	———	– –	
Cell growth ↓		– –	———	– – – –	
Wall synthesis ↓		– – –	———	– –	
Protein synthesis ↓		– – –	———	– –	
Chlorophyll content ↓			– – – – – –		
Cytochrome oxidase level ↓			– – – – – – – – –		
Nitrogen reductase level ↓			– – ———	– – –	
Stomatal Closure ↑ or ↓			– – – ———	– – –	Contrast in Species
CO_2 assimilation ↓			– – – ———	– – –	Depends on Species
Respiration ↓			– – – ———		
Proline accumulation ↑			– ———		
Cessation of all processes					
Protoplasts collapse				——— – – –	Rarely occurs
CO_2 assimilation ↔				——— – – –	under natural
Respiration ↔				——— – – –	conditions

[a] Length of the horizontal lines represents the range of H_2S dosage levels within which a process first becomes affected or a response is first noticed. Dashed lines signify deductions based on more tenuous data.
[b] With plants which received filtered air (usually charcoal filtered) as the reference point.
[c] The symbol ↑ represents an increase in that process or response, ↓ represents a decrease, and ↔ represents neither an increase nor a decrease.

reported by Steubing and Jäger (1978). Stomata may have partially closed during this process.

At this point the pattern of responses may be similar to those exhibited by plants under water stress. Growth processes, especially photosynthesis, translocation of assimilates, and respiration drop to lower levels, although the initiation of decline in these processes and the degree of decline varies with plant species and other factors. The response of cellular growth to water stress is evidenced as a slowing of shoot and root growth (Salisbury and Ross, 1978). This is usually followed closely by a reduction in cell wall synthesis and protein synthesis (Salisbury and Ross, 1978).

At slightly higher H_2S dosages protochlorophyll biosynthesis is probably inhibited and chlorophyll is converted to pheophytin. The increased activities of certain enzymes, especially GDH, GOT, and GPT, correlate with reductions in free glutamic and aspartic acid and increases in proline (Steubing and Jäger, 1978). It is unclear whether or not this process occurs to make the protoplastic osmotic potential more negative, thereby creating a greater tendency for water to move into the stressed parts. The inhibition of cytochrome oxidase may occur at this stage.

As stomata continue to close, transpiration and photosynthesis rates continue to decrease. At about this level of H_2S stress, accumulation of free proline may be found. Again this seems to be related to the H_2S-induced water stress. Salisbury and Ross (1978) stated that proline might only act as a storage pool for reduced carbon and nitrogen during stress; however, they suggest

that its presence as a solute would significantly lower the osmotic potential which could help to match the internal water potential to that of the surrounding medium.

At acute dosages of H_2S, mesophyll and epidermal cell protoplasts collapse, respiration, translocation of assimilates, and CO_2 assimilation drop to levels near zero, and leaf margins dry out becoming brittle (Meidner and Sheriff, 1976; Salisbury and Ross, 1978). Finally, it seems likely that most plants would recover if the H_2S stress was removed or lessened at any point prior to this, although growth and photosynthesis in young leaves may not reach the original rate for several days or weeks, and old leaves may be shed.

Since there is evidence of similarities between cyanide and sulfide in their inhibitory effects of cytochrome oxidase (Slater, 1950; Gassman, 1973; Nicholls, 1975; Mudd, 1979), it is logical to suggest that plants exposed to poisoning dosages of H_2S might utilize the cyanide-resistant respiration pathway of electron transport. However, the applicability here, among other things would be contingent upon the particulay species used in this study possessing this pathway. It would avoid the prevention of electron transport and poisoning of respiration. This may allow both heat and ATP to be formed at rapid rates if electron transport through the cyanide-resistant pathway was fast enough, although at considerable expense of food reserves (Salisbury and Ross, 1978). The capacity to continue tolerating toxic effects of HS^- in an unaffected manner would be determined by the biochemical threshold level (Malhotra and Hocking, 1976). However, this is mere speculation since the details

or even the absolute presence of this pathway have yet to be confirmed.

The abilty of the plant to tolerate or avoid H_2S stress may vary with plant metabolism characteristics. Plants employing the four-carbon, three-carbon dicarboxylic acid pathway of CO_2 fixation (C_4 plants) may deal with H_2S differently than those employing only the three-carbon pathway (C_3 plants). Thus, C_3-C_4 differences might offer a possibility for explanation of differences in parameter measurements. For example, the affinity for which the initial CO_2 acceptor (PEP in C_4 plants and RubP in C_3 plants) has for H_2S molecules may contribute to differences in their responses to H_2S. Ziegler (1972) reported that RubP carboxylase was inhibited by sulfite.

The relatively suppressed responses of mountain brome to the fumigations as compared to little bluestem responses may have partially resulted from inoptimal environmental growing conditions for the mountain brome plants since plant sensitivity to air contaminants is largely controlled by the environmental factors under which the plant grows (Omrod, 1978; Tibbitts, 1978). Air temperature may have been the most limiting factor in the response of mountain brome plants to the H_2S fumigations. Omrod (1978) indicates that temperature variations often affect plant sensitivity to air pollutants. Maximum chlorophyll content in mountain brome was reached at the minimum daily air temperature. Salisbury and Ross (1978) indicated that C_3 plants generally have a lower temperature optima than C_4 plants for photosynthesis and biochemical reactions.

Inoptimal temperatures for the C_3 species would slow down all growth processes (Salisbury and Ross, 1978) and its responses to H_2S fumigations, thus resulting in an insensitivity to H_2S. This would explain a lack of stimulation of growth at the low H_2S levels and a lack of suppression of growth at the high H_2S levels.

Another factor possibly affecting the magnitude of response in plant species to the H_2S fumigations may have been genetic differences. The atmospheric concentrations of sulfur-containing gases at the time of the species evolution may be partially responsible for how a plant responds to H_2S fumigations at a later evolutionary time. Björkman and Berry (1973) imply that C_4 plants may have evolved later on an evolutionary time scale than C_3 plants, therefore, through genetic selection C_4 plants may have developed different gene pools in relation to the atmospheric gas content at the time of their evolution. Bradshaw (1976) indicated that polluted situations are relatively new situations; however, the presence of sulfur-containing gases during the evolution of photosynthetic plants would most likely result in some genetically evolved adaptive significance to air contaminants by the plants. If C_3 plants did evolve during a time of higher sulfur-containing atmospheric gases than that present during the evolution of C_4 plants and did evolve some type of adaptive significance to the gases, then this could result in less variabilty in C_3 plant populations when exposed to sulfur-containing gases. Bradshaw (1976) reported evidence of plant populations that showed specific adaptations to most known pollutants including some which had been in existence for only a few

years. However, there is also widespread evidence of genetic variation in response to pollutants (Bradshaw, 1976).

SUMMARY AND CONCLUSIONS

Summary

Environmental effects of energy technology developments must be anticipated beforehand so that recommendations for environmental management policy will preclude any significant changes in ecosystems. Geothermal energy sources in Northern New Mexico may be developed in the near future. "Potential damage to vegetation and the resulting economic impact of geothermal sources should be determined before tapping such sources" (Mudd, 1979, p. 67). Few scientific studies have examined the long-term effects of a wide range of H_2S levels on vegetation under realistic field conditions. Hydrogen sulfide gas, as emitted from geothermal power plants, is an air pollutant of major concern with respect to effects on terrestial plant systems (Axtmann, 1975).

Objectives. The overall objective of this study were to determine some responses of little bluestem and mountain brome to controlled gradients of hydrogen sulfide gas under fieldenvironmental conditions in order to supplement environmental management policiy decision making with regard to the Valles Caldera Geothermal Resource Area.

Literature review. A discussion of the behavior of H_2S in the atmosphere and in plants served to identify the information needed to examine the responses. Such information included (1) a description of the chemical behavior of H_2S in the atmosphere; (2) a description of the uptake and exchange of H_2S by plants; (3) a

description of H_2S injuries to plants and their resistance to H_2S; and a review of studies relating the response of plants to H_2S fumigations.

Material and methods. Rectangular, open-top chambers for co trolled exposure of two species of plants to gradients of H_2S gas in the field were described. Experimental procedures were employe which would minimize H_2S concentration variation and which would minimize the coefficient of variation in establishing means. Analyses of variance and multiple regression methods were used to separate treatment effects and establish dose/response curves, respectively. Relationships between measured plant responses were examined. Measured responses included dry weight of topgrowth, water content of topgrowth, leaf total-nitrogen content, and leaf total-chlorophyll content.

Results. Field experiments on the effects of long-term H_2S fumigations on little bluestem and mountain brome showed the following:

1. The responses of mountain brome to the H_2S fumigations were suppressed as compared to those of little bluestem. Little bluestem dry weight showed a highly significant treatment response. At low H_2S concentrations ($\leq$ 0.34 ppm) little bluestem dry weight significantly increase Little bluestem dry weight was significantly reduced at high H_2S concentrations (1.16 ppm and higher). The regression of plant dry weight on H_2S concentratio predicted an increase of 0.023 grams dry weight with each

0.03 ppm increase in H_2S up to and including the 0.11 ppm level of H_2S and a decrease of 0.003 grams dry weight with each 0.03 ppm increase in H_2S above the 0.11 ppm level of H_2S.

Dry weight of mountain brome was relatively unaffected by the H_2S treatments until concentations reached a mean of 3.58 ppm H_2S where there was a significant ($\alpha = 0.05$) reduction in dry weight.

2. There was a significant difference in little bluestem water content treatment means, however, no discernible patterns were evident. The correlation coefficient ($r = 0.66$) indicated a significant linear relationship between dry weight and water content of topgrowth data of little bluestem at $\alpha = 0.05$. At the low H_2S levels (below 0.13 ppm) plant cells may have functioned better due to an H_2S-caused water deficit, possibly resulting in increased dry weight. Water content of mountain brome was insignificantly increased by the H_2S fumigations until concentrations reached a mean of 3.58 ppm H_2S, at which point the water content was significantly higher than that of control plants. A significant ($r = 0.43$) linear relationship between dry weight and water content of topgrowth data of mountain brome was found at $\alpha = 0.05$.

3. Little bluestem leaf-nitrogen content was significantly reduced at the 0.05-ppm level of H_2S but was insigni-

ficantly reduced at the higher H_2S levels. The relationship between leaf nitrogen content and dry weight of top growth was negative up to the 0.12-ppm level of H_2S and then positive beyond the 0.51-ppm level. The effect of the nitrogen reduction on dry weight may have been negated by other influences, especially below the 0.12-ppm level of H_2S where nitrogen content was significantly reduced. The leaf nitrogen content of mountain brome was significantly increased over that of the controls at every level of H_2S. A significant ($r = 0.76$) linear relationship between water content and nitrogen content at $\alpha = 0.05$ was found. An insignificant ($r = 0.43$) relationship between water content and dry weight at $\alpha = 0.05$ was found.

4. At low levels of H_2S (generally below 0.26 ppm) leaf chlorophyll content of little bluestem was increased after 60-and 140-h total of fumigation and reduced after 80 and 100-h total. After the initial increase in chlorophyll, a general pattern of decreasing chlorophyll content as total exposure time increased was evident. However, an increase in chlorophyll content after the 140-hour exposure time was attributed to a seven day H_2S-free "recovery" period. At the higher levels of H_2S (generally above 0.67 ppm) leaf chlorophyll content generally decreased from the 60- to 140-hour exposure time. The failure of the plants which received > 0.67 ppm H_2S to show an increase in leaf chlorophyll content after the H_2S-free "recovery" period

was attributed to irreversible plastic strain which culminated from prolonged elastic strain (Taylor, 1978). Some unmeasured variation in leaf chlorophyll content was contributed by changes in environmental factors, especially air temperature. A significant 7% reduction in leaf chlorophyll content after a four-hour fumigation was due to the H_2S fumigations. The variation in leaf chlorophyll content contributed by the spectrophotometer and its use was negligible.

Leaf chlorophyll content of mountain brome was relatively unaffected by the H_2S treatments except at the 140-h exposure time, where there was a significant increase from the 0- to the 0.37-ppm level of H_2S, and then a significant reduction from the 0.37- to the 3.58-ppm level. Mountain brome exhibited evidence of what Taylor (1978) refers to as elastic strain by recovering to produce a level of leaf chlorophyll content at the 140-h sampling time above that measured at the 60-hour sampling time. As with little bluestem, it was evident that temperature contributed considerably to the variation in leaf chlorophyll content of mountain brome. Also, the relative insensitivity of mountain brome to the H_2S fumigations may have been due to inoptimal ambient environmental growing conditions.

5. Little bluestem plants were much more sensitive to the fumigations in all measured responses than were the moun-

tain brome plants. Physiologically based hypotheses as to why this occurred were made for each measured response; however, inoptimal environmental growth conditions, especially air temperature, may have been the overriding factor.

Conclusions and Recommendations

Little bluestem. Thirty-four 4-h exposures of little bluestem to H_2S at varying concentrations produced a linear response in the stimulation of topgrowth dry weight and a curvilinear response in the reduction of topgrowth dry weight when fumigated under the experimental conditions. The following equations can be used to predict the response parameter for any similar 140-h exposure: when H_2S is $\leq$ 0.11 ppm, topgrowth dry weight of plant = $\hat{Y}$ = 0.091 + (0.77 x conc.); when > 0.11 ppm H_2S, topgrowth dry weight of plant = $\hat{Y}$ = 0.1483 + (-0.1033 x conc) + (0.02691 x $conc^2$). Seventy-five percent of the variation in response is explained by changes in H_2S concentration.

Growth of little bluestem, as dry weight of topgrowth, responded positively (0 to 95% stimulation) to low dosages of H_2S (0 to 0.11 ppm for 140 hours spread over 56 days) under the environmental conditions. This implies that geothermal H_2S emissions may be beneficial at low levels in cases of long-term intermittent fumigations. However, differences between dry weight means of control plants located in separate fumigation chambers indicated a significant source of variation other than treatment effect. At higher average H_2S levels (0.12 to 0.48 ppm) significant reduc-

tions in dry weight to the original (control) level occurred, and this reduction continued (0 to 44%) at higher average H_2S levels (0.48 to 2.39 ppm).

There was no evidence that the reduction in nitrogen content (0-38% reduction) at low H_2S levels ($\leq$ 0.11 ppm) was detrimental to plant growth. In fact, there was indication of a productivity increase at these low concentrations which may have been partially due to increases in chlorophyll synthesis (28% increase in leaf chlorophyll content) and decreases in water content (16% reduction). The relationship between dry weight production and the measured responses of chlorophyll and water content somewhat resembled a rectangular hyperbola in which dry weight production initially increased linearly with chlorophyll and water content changes and then gradually became curvilinear as other factors became limiting. The linear dependence of dry weight on chlorophyll content diminished as H_2S stress increased. Since H_2S can be used as a source of nutrient sulfur (Mudd, 1979) perhaps the productivity increase partially reflected such a usage.

The increase in leaf chlorophyll content after the seventh week (140 h) of fumigations indicated that the 7% measured reduction in leaf chlorophyll content which occurred during a 4-h fumigation period was elastic (reversible) or non-permanent at the low levels of H_2S. Thus, it can be concluded that the plants were able to recover after the 4-h fumigation by returning to a normal or above-normal rate of chlorophyll synthesis. However, this can only be said for plants after having received a seven-day fumigation-free

period since prior to that there was a general decrease in leaf chlorophyll content.

Mountain brome. Growth of mountain brome as dry weight of top-growth was relatively unaffected at average H_2S concentrations below 2.14 ppm; however,there was a reduction in growth at concentrations above 2.14 ppm and there was a significant reduction (37%) at an average H_2S concentration of 3.58 ppm. This indicates that certain grass species may have a high resitance to H_2S stress. There was evidence of very strong relationships in the direction and degree of response between nitrogen, water, and chlorophyll content for this species, however, significant increases in these responses at H_2S levels below 2.14 ppm remained hidden since they did not surface as change in dry weight.

Exposure time and air temperature interactions. There was evidence that reductions in chlorophyll content as exposure time increased was partially due to air temperature interactions; however, the variation contributed by temperature was not measured. There was also evidence that when given several days of H_2S stress-free conditions, the plant species used here had strong recovery capabilities, at least as far as chlorophyll content was concerned. Thus, the plants suffered an elastic (physiologic) strain or chlorophyll damage induced by H_2S stress. This damage was reversible in little bluestem plants which received low H_2S levels and in all mountain brome plants, but in little bluestem plants which received high H_2S levels the prolonged elastic strain was irreversible resulting in permanent damage or plastic strain. Translating these

chlorophyll content changes into changes in productivity is difficult. Due to the apparent inhibition of mountain brome responses by inoptimal environmental conditions, results obtained using little bluestem might be better used as criterion for making recommendations.

Evaluation of potential ecological and economic consequences. The H_2S dose-response functions may be important to planners of environmental control strategies because H_2S is a significant air pollutant in noncondensable gas emissions from geothermal power plants (Shinn et al., 1977). The threshold for damage was about 0.51 ppm H_2S for little bluestem; however, significant yield increases in little bluestem were realized at about 0.11 ppm. Tropospheric H_2S concentrations below 0.11 ppm would have the potential to result in economic gains in the Valles Caldera mainly through increases in animal carrying capacity, lumber production, and aesthetic values. Thus, geothermal H_2S emissions may be economically beneficial at low levels in cases of long-term intermittent fumigations.

The reduction in dry weight shown in Fig. 4 can be interpreted as stress resulting from H_2S, although it cannot necessarily be interpreted as economic loss, which depends on the particular uses of the species in the Valles Caldera. Generally, grasses are more resistant to H_2S fumigations than other plants (Shinn, 1981)[1].

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[1]Shinn, J. H., 1981. Personal communication. Environmental Sciences Division, Lawrence Livermore National Laboratory, Livermore, California.

If other more sensitive plants are affected at these concentrations, then tropospheric H_2S concentrations above 0.11 ppm would have the potential to disrupt the Valles Caldera Ecosystem. Economic losses arising from the gaseous H_2S effects might include reductions in lumber yields, animal carrying capacity, and aesthetic values. Extreme cases resulting in the elimination of native plant species or individual plants from native stands may, in turn, result in increased water runoff and soil erosion reducing the value of the land as a watershed, as a recreational area, or as wildlife habitat (Miller, et al., 1977; Benedict and Jaksch, 1979).

Reduction in growth rate may have a beneficial effect in some vegetative stands by reducing the fire hazard; however, the reverse may occur depending on several factors. Rates or direction of community succession could change, resulting in long-term ecological and economic consequences.

Level of abatement. When the H_2S fumigation results are compared to the projections of the maximum H_2S concentrations possible for the Valles Caldera Region with the operation of a 50-megawatt hydrothermal power plant (Sterns-Roger, Inc., 1975), it appears that the proposed abatement level of H_2S emission (U.S.D.O.E., 1979) would be adequate in terms of protecting native grass plants and their yields. However, numerous reservations about the concerns, or lack of them, in projecting potential H_2S concentrations in the Valles Caldera Region are discussed by Gonzales (1980).

Quality standards. Based on the H_2S fumigation results, the New Mexico ambient one-hour H_2S standard of 10 ppb was found to be too stringent. However, raising the state quality standard for H_2S is not recommended because possible synergistic effects of H_2S with other geothermal gases were not studied.

Synergistic effects of air contaminants. Since geothermal gas emissions consist of several gases other than H_2S, the effect of these gases acting in concert should be examined. However, realistic ratios of geothermal gases available to plants after settling must be considered. Also, more realistic situations of fluctuating levels of mixed pollutants should be considered.

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LITERATURE CITED

Anspaugh, L. R. and P. L. Phelps. 1978. Environmental assessment (EAR) for geothermal energy systems. Lawrence Livermore National Laboratory, Livermore, CA. Preprint no. UCRL-81910.

Axtmann, R. C. 1975. Environmental impact of a geothermal power plant. Science 187:795-803.

Bell, J. N. B. and C. H. Mudd. 1976. Sulfur dioxide resistance in plants: A case study of Lolium perenne. Pages 87-104 in Effects of Air Pollutants on Plants, T. A. Mansfield (ed.). Cambridge University Press, Cambridge.

Benedict, H. M. and W. H. Breen. 1955. The use of weeds as a means of evaluating vegetation damage caused by air pollution. Proceedings of the Third National Air Pollution Symposium. Sponsored by Stanford Research Institute, Pasadena, California, April 1955.

Benedict, H. M. and J. H. Jaksch. 1979. Economic assessement of damage to vegetation. Pages 15-1 to 15-5 in Methodology for the Assessment of Air Pollution Effects on Vegetation, W. W. Heck, S. W. Krupa, and S. N. Linzon (eds), from a specialty conference April 1978, sponsored by the Upper Midwest Section and the Technical Council of the Air Pollution Association.

Bennett, J. H. 1978. Foliar exchange of gases. In Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation. Upper Midwest Sect. Air Pollut. Control Assoc. April 1978, pp. 10-1 to 10-30.

Bennett, J. H., A. C. Hill, and D. M. Gates. 1973. A model for gaseous pollutant sorption by leaves. J. Air Pollut. Control Assoc. 23:957-62.

Björkman, O. and J. Berry. 1973. High-efficiency photosynthesis. Scien. Amer. 229:80-83.

Bradshaw, A. D. 1976. Pollution and evolution. Pages 135-160 in Effects of Air Pollutants on Plants, T. A. Mansfield (ed.). Cambridge University Press, Cambridge.

Chase, A. 1971. Manual of The Grasses of the United States (2nd Ed.). A. S. Hitchcock revision. Dover Publications Inc., New York.

Coyne, P. I. and G. E. Bingham. 1978. Photosynthesis and stomatal light responses in snap beans exposed to H_2S and O_3. J. Air Pollut. Control Assoc. 28:1119-1123.

Faller, N. 1972. Schwefeldioxid, schwefelwasserstoff, nitrose gase und ammoniak als ausschliessliche S=fzw. N-Quellen der hoheren Pflanze. Z. Planzenernahr. Dueng. Bodenk. 131:120-130.

Gassman, M. L. 1973. A reversible conversion of phototransformable protochlorophyll(ide)$_{650}$ to photoinactive protochlorophyll(ide)$_{633}$ by hydrogen sulfide in etiolated bean leaves. Plant Physiol. 51:139-145.

Gonzales, G. J. 1980. Design and evaluation of a fumigation chamber for testing potential geothermal gas emissions. M.S. Thesis. New Mexico State University, Las Cruces.

Heck, W. W. 1968. Factors influencing expression of oxidant damage to plants. Annu. Rev. Phytopath. 6:165-188.

Heck, W. W., J. A. Dunning, and I. J. Hindawi. 1965. Interactions of environmental factors on the sensitivity of plants to air pollution. J. Air Pollut. Control Assoc. 15:511-515.

Hendrickson, E. R. 1979. Hydrogen sulfide - its properties, occurrences, and uses. Pages 1-10 in Hydrogen Sulfide. National Research Council. Committee on medical and biological effects of environmental pollutants. Subcommittee on hydrogen sulfide. University Park Press, Baltimore.

Joyce, L. and R. A. Fontes. 1978. Air quality as the limiting factor on development of the Geysers Geothermal Resources. Transactions. 2:345-349.

Linzon, S. N. 1978. Effects of hydrogen sulfide on plants. Pages 149-150 in Sulfur in the Environment. Part II: Ecological Impacts, J. O. Nriagu (ed.). John Wiley & Sons, New York, Chichester, Brisbane, Toronto.

Majernik, O. and T. A. Mansfield. 1971. Effect of SO_2 pollution on stomatal movements in Vicia faba. Phytopathol. Z. 71:123-128.

Majernik, O. and T. A. Mansfield. 1972. Stomatal responses to raised atmospheric CO_2 concentrations during exposure of plants to SO_2 pollution. Environ. Pollut. 3:1-7.

Malhotra, S. S. and D. Hocking. 1976. Biochemical and cytological effects of sulphur dioxide on plant metabolism. New Phytol. 76:227-237.

McCallan, S. E. A. , A. Hortzell, and F. Wilcoxon. 1936. Hydrogen sulfide injury to plants. Contrib. Boyce Thompson Institute. 8:189-197.

Meidner, H. and T. A. Mansfield. 1968. Physiology of Stomata. McGraw-Hill Publ. Company, London.

Meidner, H. and D. W. Sheriff. 1976. Water and Plants. Blackie & Sons Limited, Glasgow and London.

Menser, H. A. and H. E. Heggestad. 1966. Ozone and sulfur dioxide synergism: Injury to tobacco plants. Science 153:424-435.

Miller, P. R., R. Kickert, O. C. Taylor, and et al. 1977. Photochemical oxidant air pollution effects on a mixed conifer forest ecosystem: A progress report. Environ. Protection Agency, Corvallis Oregon. EPA-600/3-77-104.

Mudd, B. J. 1975. Sulfur dioxide. Pages 9-22 in Responses of Plants to Air Pollutants, J. B. Mudd and T. A. Kozlowski (eds.). Academic Press, Inc., New York.

Mudd, B. J. 1979. Effects on vegetation and aquatic animals. Pages 67-80 in Hydrogen Sulfide. National Research Council. Committee on medical and biological effects of environmental pollutants. Subcommittee on hydrogen sulfide. University Park Press, Baltimore.

Nicholls, P. 1975. The effect of sulphide on cytochrome aa3. Isoteric and allosteric shifts of the reduced α peak. Biochem. Biophys. Acta 396:24-35.

Omrod, D. P. 1978. Pollution in Horticulture. Fundamental aspects of pollution control and environmental science: 4. Elsevier Scientific Publishing Co., Amsterdam.

Salisbury, F. B. and C. L. Ross. 1978. Plant Physiology (2nd edition). Wadsworth Publishing Company, Inc., Belmont, CA.

Shinn, J. H., B. R. Clegg, M. L. Stuart, and S. E. Thompson. 1976. Exposures of field-grown lettuce to geothermal air pollution: photosynthetic and stomatal response. J. Environ. Sci. Health. A11(10&11):603-612.

Shinn, J. H., B. R. Clegg, and M. L. Stuart. 1977. A linear-gradient chamber for exposing field plants to controlled levels of air pollutants. Lawrence Livermore National Laboratory, Livermore, CA. Report no. UCRL-80411.

Shinn, J. H. and J. R. Kercher. 1978. Ecological effects of atmospheric releases from synthetic fuels processes. Lawrence Livermore National Laboratory, Livermore, CA. Report no. UCRL-81691.

Slater, E. C. 1950. The components of the dihydrocozymase oxidase system. Biochem. J. 46:484-499.

Smith, R. P. 1979. Absorption, distribution, metabolism, and excretion of sulfides in animals and humans. Pages 21-24 in Hydrogen Sulfide. National Research Council. Committee on medical and biological effects of environmental pollutants. Subcommittee on hydrogen sulfide. University Park Press, Baltimore.

Sprung, J. L. 1976. Tropospheric oxidation of H_2S. Advances in Environ. Sci. and Technol.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc.

Sterns-Roger Inc. 1975. Preliminary ground-level H_2S concentration estimates, Baca Location No. 1, New Mexico. Environmental Sciences Division. Prepared for Geothermal Division, Union Oil Company of California. Project no. C-15474.

Steubing, L. and H. J. Jäger. 1978. Ecophysiological-biochemical effect of H_2S on Pisum sativum L. Angewandte Botanik. 52:137-148.

Taylor, G. E. Jr. 1978. Plant and leaf resistance to gaseous air pollution stress. New Phytol. 80:523-534.

Thompson, R. C. 1976. Behavior of H_2S in the atmosphere and its effects on vegetation. U. S. Dept. Commerce NTIS report no. NSF-RA-760398. Prepared for NSF, Washington, D. C.

Thompson, R. C. and G. Kats. 1978. Effects of continuous H_2S fumigation on crop and forest plants. Environ. Sci. Technol. 12:550-553.

Thornton, N. C. and C. Setterstrom. 1940. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulfhur dioxide gases. III. Green plants. Contrib. Boyce Thompson Institute 11:343-356.

Tibbitts, T. W. 1978. Monitoring. Pages 3-1 to 3-9 in Methodology for the Assessment of Air Pollution Effects on Vegetation, W. W. Heck, S. V. Krupa, and S. N. Linzon (eds.). From a specialty conference April 1978. Sponsored by the Upper Midwest Section and the Technical Council of the Air Pollution Association.

United States Department of Energy. 1979. Draft environmental impact statement; geothermal demonstration program; 50 MWe power plant; Baca Ranch, Sandoval and Rio Arriba counties, New Mexico. Report no. DOE/EIS-0049-D.

Unsworth, M. H., P. V. Biscoe, and H. R. Pinckney. 1972. Stomatal responses to sulphur dioxide. Nature. 239:458-459.

Unsworth, M. H., P. V. Biscoe, and V. Black. 1976. Analysis of gas exchange between plants and polluted atmospheres. Pages 5-30 in Effects of Air Pollutants on Plants, T. A. Mansfield (ed.). Cambridge University Press, Cambridge.

Vernon, L. P. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32:1144.

Wellburn, A. R., T. M. Capron, H. -S. Chan, and D. C. Horsman. 1976. Biochemical effects of atmospheric pollutants on plants. Pages 105-114 in Effects of Air Pollutants on Plants, T. A. Mansfield (ed.). Cambridge University Press, Cambridge.

Winner, W. E. and H. A. Mooney. 1980. Ecology of SO_2 resistance: II. Photosynthetic changes of shrubs in relation to SO_2 absorption and stomatal behavior. Oecologia (Berl). 44:296-302.

Ziegler, I. 1972. The effects of $SO_3^{=}$ on the activity of ribulose 1,5-diphosphate carboxylase in isolated spinach chloroplasts. Planta. 103:155-163.

APPENDIX TABLES

Appendix Table 14. ANOVA for dry weight of little bluestem fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	12	0.12924	0.01077	19.23**
Within Trts.	85	0.04760	0.00056	
TOTAL	97	0.17684		

**Highly significant

Appendix Table 15. ANOVA for dry weight of mountain brome fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	11	0.01971	0.00179182	4.93*
Within Trts.	86	0.03126	0.00036339	
TOTAL	97	0.05097		

*Significant

Appendix Table 16. ANOVA for water content of little bluestem topgrowth data fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Treatments	11	1550.8	140.98	10.62**
Within Treatments	62	822.8	13.27	
TOTAL	73	2373.6		

**Highly Significant

Appendix Table 17. ANOVA for water content of mountain brome topgrowth data for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	11	334.8	30.4	3.17*
Within Trts.	87	831.5	9.6	
TOTAL	98	1166.3		

*Significant

Appendix Table 18. ANOVA for leaf nitrogen content data of little bluestem fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	8	2.050	0.25625	2.11[NS]
Within Trts.	18	2.184	0.12133	
TOTAL	26	4.234		

[NS]Not Significant

Appendix Table 19. ANOVA for leaf nitrogen content data of mountain brome fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	11	6.394	0.58127	7.74*
Within Trts.	72	5.410	0.07514	
TOTAL	83	11.804		

*Significant

Appendix Table 20. ANOVA for leaf chlorophyll content data of little bluestem fumigated for 60 hours.

Source of Variation	df	SS	MS	F
Among Trts.	8	0.528	0.066	2.75*
Within Trts.	14	0.333	0.024	
TOTAL	22	0.861		

*Significant

Appendix Table 21. ANOVA for chlorophyll content data of little bluestem leaves fumigated for 80 hours.

Source of Variation	df	SS	MS	F
Among Trts.	6	0.223	0.037	3.36*
Within Trts.	19	0.215	0.011	
TOTAL	25	0.438		

*Significant

Appendix Table 22. ANOVA for chlorophyll content data of little bluestem leaves fumigated for 100 hours.

Source of Variation	df	SS	MS	F
Among Trts.	8	0.414	0.05175	2.16^{NS}
Within Trts	16	0.385	0.024	
TOTAL	24	0.799		

^{NS}Not Significant

Appendix Table 23. ANOVA for chlorophyll content data of little bluestem leaves fumigated for 140 hours and for spectrophotometer sampling error.

Source of Variation	df	SS	MS	F
H_2S Trts.	8	1.0457	0.1307	2.90*
Analysis within H_2S Trts. = Experimental Error	13	0.001	7^{-5}	0.002^{NS}
Sampling Error (Spectrophotometer)	48	2.162	0.04504	
TOTAL	69	3.2087		

*Significant

^{NS}Not Significant

Appendix Table 24. ANOVA for chlorophyll content data of mountain brome leaves fumigated for 60 hours.

Source of Variation	df	SS	MS	F
Among Trts.	4	0.0224	0.0056	0.03[NS]
Within Trts.	31	0.6095	0.1966	
TOTAL	35	0.6319		

[NS]Not Significant

Appendix Table 25. ANOVA for chlorophyll content data of mountain brome leaves fumigated for 80 hours.

Source of variation	df	SS	MS	F
Among Trts.	4	0.0044	0.0011	0.08[NS]
Within Trts.	32	0.8945	0.0133	
TOTAL	36	0.8989		

[NS]Not Significant

Appendix Table 26. ANOVA for chlorophyll content data of mountain brome leaves fumigated for 120 hours.

Source of Variation	df	SS	MS	F
Among Trts.	11	0.4494	0.04085	2.19*
Within Trts.	74	1.3780	0.01862	
TOTAL	85	1.8274		

*Significant

Appendix Table 27. ANOVA for chlorophyll content data of mountain brome leaves fumigated for 140 hours.

Source of Variation	df	SS	MS	F
Among Trts.	10	0.8443	0.08443	9.98**
Within Trts.	73	0.6175	0.00846	
TOTAL	83	1.4618		

**Highly Significant

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