

THE EFFECTS OF ATMOSPHERIC SULFUR DIOXIDE  
AND BISULFITE CONTAINING SOLUTIONS ON FOUR  
ST. AUGUSTINEGRASS (Stenotaphrum secundatum  
(Walt.) Kuntze) CULTIVARS

A Thesis

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## ABSTRACT

The Effects of Atmospheric Sulfur Dioxide and Bisulfite Containing Solutions on Four St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze) Cultivars. (December 1980)

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Four cultivars (Floritam, Raleigh, Seville, and Texas Common) of St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze) were exposed to  $0.20 \mu\text{l liter}^{-1} \text{SO}_2$  ( $4 \text{ h day}^{-1}$ , 5 days  $\text{week}^{-1}$ ) for 5 weeks. None of the cultivars were visually injured, nor were vertical canopy growth rate, stolon internode elongation, or leaf blade chlorophyll content affected by the end of the 5-week period. Dry matter accumulation was reduced 15% (compared to control plants) for the Floritam plants exposed to the 5-week  $\text{SO}_2$  fumigation, while dry weight accumulation was not affected for the other three cultivars.

Additional plants of the same four cultivars were exposed to  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$  for four consecutive days ( $10 \text{ h day}^{-1}$ ). Floritam was significantly (5% level) more injured than Texas Common, which was significantly (5% level) more injured than either Raleigh or Seville.

Leaf sections of the same four cultivars were exposed to solutions containing  $\text{KHSO}_3$  for 2 h. The  $\text{KHSO}_3$  exposures were used as a model of an  $\text{SO}_2$ -plant interaction. Following the  $\text{KHSO}_3$

exposures, the leaf sections were rinsed and placed in flasks which were subsequently sealed to prevent gas exchange. Gas samples were drawn from the flasks at various times and analyzed for ethylene and ethane concentration by gas chromatography. The results of the hydrocarbon analysis indicated that ethylene is not a good measure of  $\text{KHSO}_3$  injury. Ethane production rates, however, correctly predicted that St. Augustinegrass is resistant to  $\text{SO}_2$ -induced injury, and that Floratam and Texas Common are more susceptible to injury than are Raleigh and Seville. This was borne out by the results of the acute  $\text{SO}_2$  fumigation portion of this study.

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## INTRODUCTION

Sulfur dioxide ( $\text{SO}_2$ ) is a major phytotoxic air pollutant throughout the industrialized world. While  $\text{SO}_2$  is a naturally occurring component of the atmosphere ("clean" air contains 0.002-0.008  $\mu\text{l liter}^{-1}$   $\text{SO}_2$ ), man's activities (primarily the combustion of fossil fuels) have often elevated the atmospheric  $\text{SO}_2$  concentrations to phytotoxic levels (23,43).

While  $\text{SO}_2$  can injure all plants investigated at high concentrations, there exists significant diversity in susceptibility to injury among species (19,27,35) and even among many cultivars within the same species (7,10,12,26,28,35). Even though the effects of  $\text{SO}_2$  on higher plants have been well documented for many species (27), the exact mechanisms of injury to plants have not been elucidated, nor are resistance mechanisms completely understood (19).

The effects of  $\text{SO}_2$  on St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze), a major "warm-season" turfgrass in the United States, have not been reported in the literature. Because St. Augustinegrass is extensively grown in many of the  $\text{SO}_2$  polluted urban and industrial areas of the Southern United States (6), the ability to select  $\text{SO}_2$  resistant cultivars would be of benefit.

Presently, screening procedures for  $\text{SO}_2$  resistance in

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The style and format of this thesis are those of Plant Physiology.

turfgrasses (as well as other plant species) involve expensive and time consuming fumigations of large numbers of whole plants (10,28). In addition to the inconvenience and expense of  $\text{SO}_2$  resistance screening, results are generally based on visual (possibly bias) rankings and ratings. Quantitative, objective measurement of plant injury eliminates judgement bias when evaluating species and/or cultivars for  $\text{SO}_2$ -induced injury resistance or susceptibility. Also, results obtained by different investigators can be compared when utilizing quantitative measurement of injury.

Ideally, screening processes would not involve large, whole plant systems, but instead, be accomplished using excised plant parts, such as leaf discs. In fact, such a system is being used to a limited extent today. Briefly, it involves the exposure of leaf discs to solutions containing a salt of the bisulfite ( $\text{HSO}_3^-$ ) ion, the phytotoxic product of atmospheric  $\text{SO}_2$  (36,43), followed by the subsequent quantitative measurement of ethylene and/or ethane production by the leaf discs (11). With the use of this system, significantly larger populations can be evaluated, and the evaluations can be conducted rapidly.

Screening St. Augustinegrass cultivars for  $\text{SO}_2$  resistance will help assure better maintenance of urban greenery. A healthy, vigorous turf significantly reduces the cost of controlling unwanted diseases, weeds, and insects, and increases its tolerance to environmental stress (6).

With these points in mind, the objectives of this study were formulated as follows: a) to determine if the St. Augustinegrass species is susceptible to  $\text{SO}_2$ -induced injury during realistic chronic exposures; b) to determine if cultivar differentials for  $\text{SO}_2$  resistance exists within the St. Augustinegrass species; c) to evaluate the first two objectives using quantitative as well as qualitative methods; and d) to determine if the "bisulfite/ethylene-ethane system" is applicable in screening St. Augustinegrass germplasm sources for resistance to  $\text{SO}_2$ -induced injury.

## LITERATURE REVIEW

While  $\text{SO}_2$  can be extremely damaging to higher plants (26), not all plants are injured by moderate  $\text{SO}_2$  exposures (35). Many plants, in fact, benefit from low-level exposures to  $\text{SO}_2$  (8,13,14, 15,25,27,43). Sulfur is an essential element for plant growth and development. Plants growing in sulfur deficient soils can utilize atmospheric sulfur for metabolic requirements, such as the formation of sulfur containing proteins and nucleic acids (13,27,35). Such utilization of  $\text{SO}_2$  has been reported to increase yields of perennial ryegrass (Lolium perenne) (9,13). However, when a plant is exposed to more  $\text{SO}_2$  than it can metabolize, it is susceptible to injury (9,25,35,43). For many years, the threshold concentration for  $\text{SO}_2$ -induced injury to plants has been set at  $0.15 \mu\text{l liter}^{-1}$  (34).

Symptoms of  $\text{SO}_2$ -induced Injury

Typical symptoms of an acute exposure to  $\text{SO}_2$  are general interveinal chlorosis and necrosis of the leaf blade, with the necrosis ranging in color from brown to white (27). Chronic injury is generally thought of as accelerated senescence (19,35). For grasses in particular, chronic injury is characterized by an overall yellowing of the leaves, while acute injury usually involves a white or tan necrosis on the apical portion of the leaf blades (7,10,28). Detailed descriptions of injury by  $\text{SO}_2$ , as well as other air pollutants, have been published by Thomas (33).

For many years,  $\text{SO}_2$  has been implicated in producing what is called "invisible injury" (34). Although some evidence exists to support the contrary (13,34,35), it is thought by many that photosynthesis can be inhibited, early senescence can be realized, and vigor can be reduced in plants exposed to chronic  $\text{SO}_2$  levels (9,19,20,34,35). This is thought to be true even when visible symptoms are not immediately apparent; thus making it difficult to assess the true extent of  $\text{SO}_2$  damage. While gross injury symptoms may not be evident, many investigators believe more subtle injury, and subsequent crop damage, can be significant (25).

While no data are available in the literature for St. Augustinegrass, many plant species have been shown to exhibit cultivar differentials for  $\text{SO}_2$ -induced injury resistance (7,10,12, 26,28,35). When visually rated after an acute  $\text{SO}_2$  exposure, cultivar discrepancy can range from minor lesions to complete destruction of the exposed tissue.

#### Effects of $\text{SO}_2$ on Chl

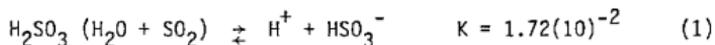
The effects of  $\text{SO}_2$  on Chl have been well documented for many species (7,19,24,25,27,32). Sulfur dioxide exposure resulted in the breakdown of Chl to phaeophytin and  $\text{Mg}^{2+}$  ions for both lichens and bryophytes when exposed to acute ( $2-5 \mu\text{l liter}^{-1}$ ) doses (25). It was also found that chronic (less than  $0.15 \mu\text{l liter}^{-1}$ )  $\text{SO}_2$  exposure reduced the Chl content of  $\text{SO}_2$  sensitive lichens and mosses (25) and perennial ryegrass (7), while not affecting the Chl content of resistant species and cultivars. It has been

reported that Chl a is more sensitive to breakdown by  $\text{SO}_2$  than is Chl b (25).

In a comprehensive study of both a  $\text{SO}_2$  susceptible and resistant perennial ryegrass cultivar (7), it was found that shoot dry weights of the susceptible cultivar (S23) were 40% less than control plants, following a 9-week exposure to  $0.14 \mu\text{l liter}^{-1} \text{SO}_2$ , while the shoot dry weights of the resistant cultivar (Helmshore) were not affected. Of other parameters investigated (leaf sulfur content, stomatal resistance, stomatal length, stomatal density, membrane permeability, and leaf Chl content), only the Chl content differed for the two cultivars during or after the chronic  $\text{SO}_2$  exposures. The  $\text{SO}_2$  exposures implemented in the study reduced the Chl content (expressed on a mass per leaf area basis) of the S23 plants by about 25% while the Chl content in the leaves of the Helmshore plants were not affected by the  $\text{SO}_2$  fumigation.

#### Chemistry of $\text{SO}_2$ -plant Interactions

To understand the interactions between  $\text{SO}_2$  and plant tissue, the chemistry of  $\text{SO}_2$  must be considered. Sulfur dioxide is very soluble in water, and when in solution on or in a plant, rapidly establishes the following equilibria (25):



The  $\text{H}_2\text{SO}_3$  species has never been isolated (32) and should probably be thought of as  $\text{SO}_2$  in water (27). The relative amount of  $\text{HSO}_3^-$

and  $\text{SO}_3^{2-}$  in plant tissue is pH dependent (Fig. 1).

The bisulfite ion ( $\text{HSO}_3^-$ ) is generally thought to be the phytotoxic product of  $\text{SO}_2$  after it has entered the plant (19,36,43). Because of this, excised leaves and leaf discs have been exposed to a solution containing a salt of the bisulfite ion, instead of exposing whole plants to  $\text{SO}_2$ , when studying species and cultivar resistance differentials (11,25). Larger populations can be effectively tested using this "bisulfite system".

#### Ethylene and Ethane Production by $\text{SO}_2$ -injured Tissue

The production of ethylene and ethane from excised leaves exposed to solutions containing bisulfite has been well correlated with visible injury as a result of acute exposures of whole plants to  $\text{SO}_2$  (11). It is important to consider that elevated ethylene production occurs in cells that have been physiologically perturbed (stressed), but not decompartmentalized (1,31), while "ethane production is not dependent on intact compartmentalisation in the leaf cells, but rather depends on cellular disorder," that is, death (18).

In preliminary work, the author has determined that excised St. Augustinegrass leaves are moderately injured (40% of the surface area) by a 10 h exposure to a 10 mM  $\text{NaHSO}_3$  solution, and severely injured (90+% of the surface area) by a 10 h exposure to a 50 mM  $\text{NaHSO}_3$  solution. During these same investigations, it was determined that excised St. Augustinegrass leaves were not visibly injured (<5% of the surface area) by equivalent exposures to either

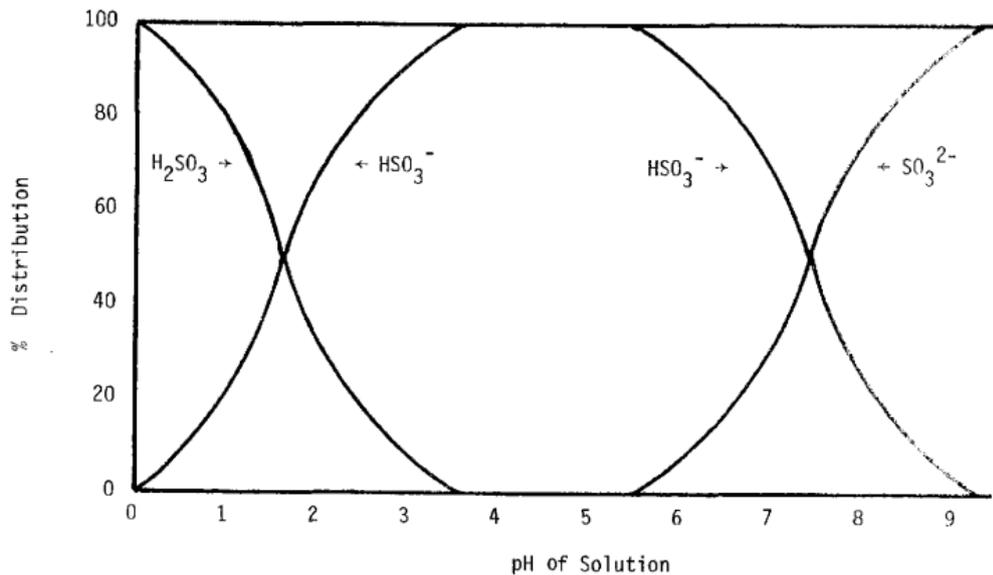


Fig. 1. Relative amount of sulfite species as a function of solution pH. Adapted from Puckett *et al.* (32).

10 mM or 50 mM  $K_2SO_4$  solutions (unpublished data). No data are available in the literature on the effects of  $HSO_3^-$  exposures on ethylene and ethane production from *St. Augustinegrass*.

In addition to ethylene and ethane studies, bisulfite has been reported to rapidly destroy spinach (*Spinacia oleracea*) Chl in vitro (29,30). Results of in vivo and in vitro lichen-pigment, in vitro spinach Chl, and in vitro pine (*Pinus contorta*) Chl studies demonstrate that aqueous  $SO_2$  ( $HSO_3^-$  and  $SO_3^{2-}$ ) effectively degrades Chl (24,32). Chl a is destroyed more readily by  $HSO_3^-$  than is Chl b (24,29,30,32) which also has been shown to be true for  $SO_2$ .

#### Evaluation of $SO_2$ -induced Injury

Visible injury to a plant following an acute  $SO_2$  fumigation can be rapidly and inexpensively evaluated by visual ratings. However, observer bias when scoring air pollution injury can be a problem (38), especially when comparing results from one study to another (22). Quantitative measurement can eliminate this problem. Air pollution injury to plants has been quantitatively assessed by measuring leaf Chl concentration (7,16,22) as well as ethylene and ethane production (1,11,17,31). These methods are important in assaying  $SO_2$  injury because: a)  $SO_2$  in high atmospheric concentrations may cause the destruction of, or the inhibition of biosynthesis of, Chl in susceptible plants while not affecting Chl concentrations in resistant plants (7,25,27); b) ethylene is produced at elevated rates by  $SO_2$  and  $HSO_3^-$  injured plant tissue (11,31,40) and quantitative measurement of ethylene has been

correlated with differential cultivar susceptibility to  $\text{SO}_2$ ,  $\text{HSO}_3^-$ , and  $\text{O}_3$  (11,17,37); and c) ethane production is a good measure of tissue that has been killed, while ethylene is a good measure of tissue that has been stressed, but not killed (1,2,31).

#### Summary

The current literature supports several concepts. One, there is significant diversity in susceptibility to  $\text{SO}_2$  injury among plant species. Two, there is often also significant diversity in susceptibility to  $\text{SO}_2$  injury among cultivars within a plant species. Three, low-level  $\text{SO}_2$  exposures may or may not be detrimental to plant growth and development. Four, plants can benefit from low-level  $\text{SO}_2$  exposures. Five, the degradation of Chl by  $\text{SO}_2$  may be a major link in the chain of events leading to injury following a  $\text{SO}_2$  fumigation. Six, bisulfite and sulfite ions are the phytotoxic products of  $\text{SO}_2$  within a plant. Seven, the "bisulfite/ethylene-ethane system" can be used to rapidly and quantitatively study the effects of  $\text{SO}_2$  on plants. This research was designed to determine if and how these concepts apply to the St. Augustinegrass species.

## MATERIALS AND METHODS

## Plant Material and Growth Conditions

During June 1980, plugs of four St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) cultivars (Floratum, Raleigh, Seville, and Texas Common) were collected, potted, and transported to the first of three identical, controlled-environment growth chambers (1.1 × 2.2 × 1.1 m). The plugs (5.5 cm diameter by 6.0 cm depth, soil dimensions) were collected from two-year-old plots grown on a modified sand (92-93% sand, 5% silt, 2-3% clay) soil at the Texas A&M University Turfgrass Field Laboratory (College Station, TX) and then potted in 850-ml plastic pots (11 cm top diameter, 8 cm bottom diameter) containing the same modified sand soil. Five holes (3 mm diameter) were burned into the bottom of each pot to facilitate drainage and the leaching of any accumulated salts.

The environmental conditions in the growth chambers were as follows: quantum flux density at canopy height,  $210 \mu\text{E m}^{-2} \text{s}^{-1}$  (PAR) supplied by a mixture of cool-white fluorescent and incandescent lamps and measured with a Lambda Instruments quantum sensor and meter (model LI-185); photoperiod, 12 h light/12 h dark (light period, 8 AM to 8 PM); temperature, 31 to 34 C light/24 to 26 C dark; CO<sub>2</sub> concentration and humidity were not controlled. Air entering the growth chambers was vented from outside the building and passed through a charcoal filter. Air

in the chambers was continuously renewed.

For the months of June and July, the plants in each pot were watered with Hoagland's solution 1 (21) on Mondays, Wednesdays, and Fridays (100 ml pot<sup>-1</sup>), and with distilled water on Saturday and Sunday (100 ml pot<sup>-1</sup>). Beginning with the first week in August, the plants in each pot were watered with a solution that was  $1.9(10)^{-3}$  M for  $(\text{NH}_4)_2\text{SO}_4$  and  $2.5(10)^{-5}$  M for EDTA-chelated-Fe at 9:30 AM Monday through Friday (100 ml pot<sup>-1</sup>), and with distilled water on Saturday and Sunday (100 ml pot<sup>-1</sup>). This irrigation regime was sufficient to flush water through the root zone daily.

Prior to the initiation of the chronic SO<sub>2</sub> exposure period (see below), all the turfs were cut at a height of 7.5 cm each Saturday. All plant parts extending beyond the perimeter of each pot were also cut at this time. To prevent the possibility of masking any symptoms of SO<sub>2</sub> injury during the chronic exposure period, the turfs used in the chronic SO<sub>2</sub> exposure portion of these investigations were not cut after initiation of that phase of this study. No pesticides were applied during any portion of these investigations.

#### Chronic SO<sub>2</sub> Exposure

On August 20, five reps of each cultivar were moved to the second of the three growth chambers for fumigation with SO<sub>2</sub>. Five reps of each cultivar were used as controls and remained in the first chamber. All other plants were moved to the third chamber.

The SO<sub>2</sub> was metered into the charcoal-filtered air entering

the fumigation (second) chamber as illustrated by Figure 2. The  $\text{SO}_2$  was metered from a tank of  $10 \text{ ml liter}^{-1}$   $\text{SO}_2$  balanced with nitrogen and obtained from Air Products and Chemicals (La Porte, TX). The  $\text{SO}_2$  exposures ( $0.20 \pm 0.02 \mu\text{l liter}^{-1}$ ) were commenced at 10 AM and terminated 4 h later, at 2 PM, Monday through Friday for five weeks, beginning August 25. The  $\text{SO}_2$  concentrations within the chamber were continuously monitored with a Meloy Laboratories (Springfield, VA) portable sulfur gas analyzer (model SA 165). The gas analyzer was zeroed with charcoal-filtered air each morning prior to the  $\text{SO}_2$  fumigation. Air was sampled from the center of the growth chamber at canopy height through Teflon tubing ( $270 \text{ ml min}^{-1}$ ).

Before and during the chronic  $\text{SO}_2$  exposure period, the vertical growth rates of the four cultivars were monitored as described below. At the termination of the chronic  $\text{SO}_2$  exposure period, stolon internode elongation, leaf blade Chl content, and dry weight accumulation were determined for each of the four cultivars for both the  $\text{SO}_2$  fumigated and control plants (see below).

#### Vertical Growth Rate Analysis

The canopy height (soil to the highest point of the canopy) was measured when physically supporting the leaves in a vertical position. Measurements were made each Saturday. The vertical growth rate ( $\text{mm day}^{-1}$ ) was calculated by dividing the change in canopy height from the previous Saturday by 7 days.

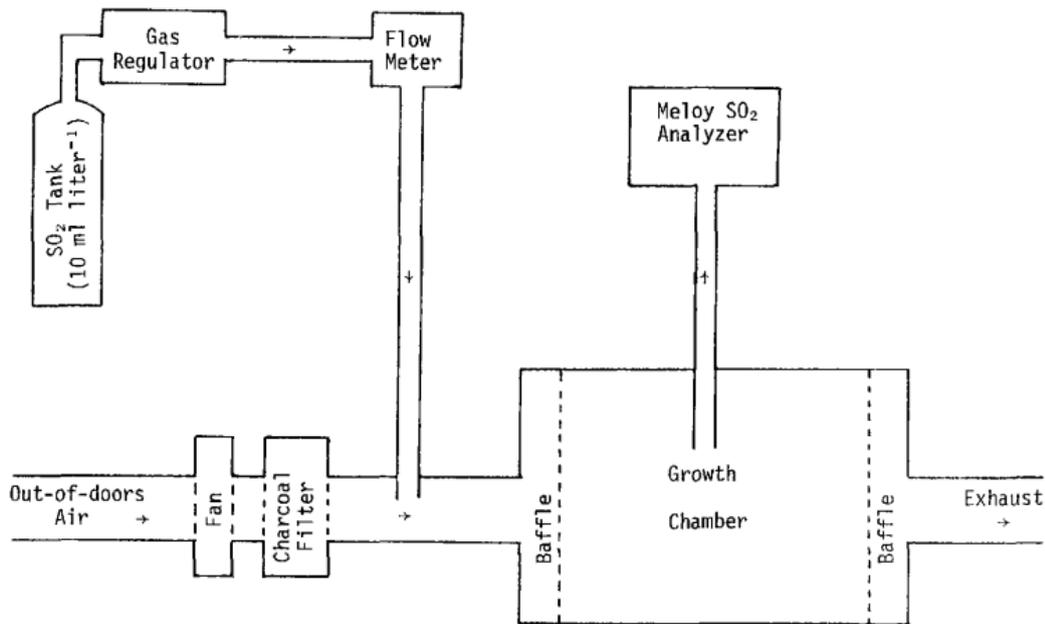


Fig. 2. Sulfur dioxide exposure and analysis system. The MeIoy  $\text{SO}_2$  analyzer sampled air from the center of the growth chamber at canopy height. All tubing was composed of Teflon. Not to scale.

### Stolon Internode Elongation Measurement

At the termination of the chronic SO<sub>2</sub> exposure period, internode lengths were measured for all the stolons extending beyond the perimeter of the pots of both the SO<sub>2</sub> fumigated and control plants. The stolons extending beyond the perimeter of the pots represented growth during the fumigation period.

### Chl Extraction and Measurement

Chls a and b were extracted from expanded leaf blade tissue by soaking in 95% ethanol and measured spectrophotometrically by the method of Knudson et al. (22) which was modified as follows: Leaf blade tissue was placed in 110-ml brown jars. About 50 ml of ethanol was added to each jar. The jars were capped and placed in the dark for 24 h. At the end of the 24 h period, the ethanol was decanted into a 250-ml volumetric flask which was stoppered and placed in the dark. Ethanol (50 ml) was again added to the jar and the tissue soaked for an additional 24 h period. The ethanol was decanted into the same volumetric flask and the procedure repeated two more times. Following the fourth soaking period, the jar and the leaf tissue were rinsed with additional ethanol which was added to the flask. The extract was brought to volume with ethanol. Following extraction, the leaf tissue was dried in a forced air oven and weighed.

Aliquots of each Chl extract were then measured spectrophotometrically in 1 cm diameter tubes using a Bausch & Lomb

Spectronic 20. Absorbances were measured at 649 and 665 nm. The absorbance readings were converted to Chl concentrations by utilizing the following equations (39):

$$\frac{\mu\text{g Chl a}}{\text{ml solution}} = ((13.70)(A_{665 \text{ nm}})) - ((5.76)(A_{649 \text{ nm}})) \quad (3)$$

$$\frac{\mu\text{g Chl b}}{\text{ml solution}} = ((25.80)(A_{649 \text{ nm}})) - ((7.60)(A_{665 \text{ nm}})) \quad (4)$$

where A is the absorbance using an ethanol (95%) blank.

#### Dry Weight Accumulation Determination

After leaf blades had been removed for Chl extraction (about one half the blades from each pot were used) following the chronic SO<sub>2</sub> exposure period, the leaf sheaths and remaining leaf blades (expanded and unexpanded) were removed from all the plants in each pot and dried in a forced air oven and weighed. The dry weight of the leaf blades used for Chl extraction was added to the dry weight of the remaining leaf blades and sheathes for each pot.

#### Acute SO<sub>2</sub> Exposure

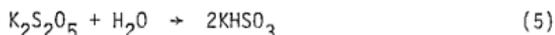
Three reps of each of the four cultivars, which had not been previously exposed to any SO<sub>2</sub>, were exposed to 1.0 ± 0.1 μl liter<sup>-1</sup> SO<sub>2</sub> for 10 h, from 10 AM to 8 PM, during four consecutive days. Pots of each cultivar used as controls were not exposed to SO<sub>2</sub>. After allowing time for symptoms of injury to develop (20 h after termination of the SO<sub>2</sub> fumigation on the fourth day), each pot was visually rated for injury (0-no injury, 9-severe injury),

injury symptoms were noted and photographed, and Chl was extracted from the leaf blades of both the SO<sub>2</sub> fumigated and control plants as previously described.

#### Bisulfite Ion Treatment

Leaf sections of each of the four cultivars were exposed to bisulfite containing solutions. Sections (2 cm long) were cut from approximately the middle of the leaf blades of the three oldest leaves of randomly selected five-leafed nodes (3 sections leaf<sup>-1</sup>). After being cut, the surface area (one side) of the leaf sections was determined and the sections were halved by cutting along the midrib. The leaf sections were then immediately placed (floated) in 250-ml beakers containing a buffered (pH 4.2-4.3) solution of either 10 or 50 mM KHSO<sub>3</sub>, or 10 or 50 mM K<sub>2</sub>SO<sub>4</sub> (controls). One half of each leaf section (2 cm) was floated on a KHSO<sub>3</sub> solution while the other half was floated on the corresponding K<sub>2</sub>SO<sub>4</sub> solution. Half of the leaf sections in each beaker were floated adaxial side up with the other half floated abaxial side up. All the treatments (KHSO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub>) were initiated at about 10 AM and terminated 2 h later. Treatments were conducted in one of the growth chambers.

The KHSO<sub>3</sub> used was formulated as K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> granules. When dissolved in water, K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> is completely converted to KHSO<sub>3</sub> as follows:



Upon termination of the treatment, the leaf sections were thoroughly rinsed on a fiberglass screen with distilled water to remove ions from the tissue surface. After rinsing, the leaf sections were placed in 50-ml erlynmeyer flasks containing distilled water (5 ml) and chloramphenicol to suppress bacterial activity ( $50 \mu\text{g ml}^{-1}$ ). The flasks were sealed with serum bottle stoppers. After sealing, the flasks were placed under a fluorescent lamp ( $30 \mu\text{E m}^{-2} \text{s}^{-1}$ , PAR) which was continuously on. Following the gas sampling period (see next paragraph), the percent injury to the leaf sections was visually estimated, and the Chl was extracted using the procedure described above with smaller ethanol volumes. The four treatments were replicated three times for each cultivar.

#### Ethylene and Ethane Measurement

Gas samples (1.0 ml) were drawn with a gas-tight syringe from each of the sealed flasks every 12 h, for 48 h, following the  $\text{KHSO}_3$  and  $\text{K}_2\text{SO}_4$  treatments. The gas samples were immediately injected into a Beckman GC 72-5 gas chromatograph equipped with a 3.2 mm  $\times$  1.8 m alumina column and a flame ionization detector. Chromatograph temperatures were: inlet, 75 C; column, 80 C; and detector, 150 C. Flow rates of the gases used were: carrier (helium),  $70 \text{ ml min}^{-1}$ ; hydrogen,  $36 \text{ ml min}^{-1}$ ; and air,  $300 \text{ ml min}^{-1}$ . Peaks were identified based on retention time. Ethane appeared first. Ethylene and ethane concentrations in

the gas samples were calculated by measuring the peak heights as recorded by a Fisher Recordall (series 5000).

Standard curves (peak height vs. concentration) were determined for each gas twice before the gas sampling began. Gas samples of known ethylene and ethane concentration were injected into the gas chromatograph during the chromatography periods to determine the efficiency of the chromatograph. Ambient air samples were also chromatographed to ascertain the amount of ethylene and ethane, if any, contaminating the syringe.

#### Statistical Tests

The standard deviation (SD) was calculated for all quantitative measurements by utilizing the following equation:

$$SD = \left( \frac{\sum (x_i - \bar{x})^2}{n} \right)^{1/2} \quad (6)$$

A Duncan's multiple range test was conducted for the visual ratings following the acute SO<sub>2</sub> exposures.

## RESULTS AND DISCUSSION

### Plant Material and Growth Conditions

St. Augustinegrass is recognized as being an inefficient user of soil supplied iron. This often results in the development of interveinal chlorosis of the leaf blades (6). Watering the St. Augustinegrass plants with Hoagland's solution number 1 ( $8.8(10)^{-6}$  M for chelated Fe) was not adequate in supplying iron, which was manifested in the development of chlorosis in all four cultivars. At the end of July, the use of the Hoagland's solution was terminated. Instead, the amount of iron supplied to the plants (EDTA chelated) was increased by 180%. Also,  $(\text{NH}_4)_2\text{SO}_4$  was added to the irrigation water at a high rate (see page 12) to a) lower the soil solution pH and thus make the iron present more "plant available", b) to provide enough nitrogen for optimal plant growth, and c) to assure a high level of soil supplied sulfur. Within one week of the initiation of the new irrigation regime, the chlorosis had disappeared in all four cultivars. No other problems with the plant material or growth conditions were evident at any time during the course of the investigations.

### Chronic $\text{SO}_2$ Exposure

#### Selection of Concentrations and Durations of Exposure

During 1979 and the first six months of 1980, the Texas Air

Control Board recorded 3-h average  $\text{SO}_2$  concentrations in excess of  $0.30 \mu\text{l liter}^{-1}$  in the Corpus Christi and Houston regions; a 3-h average concentration of  $0.25 \mu\text{l liter}^{-1}$  in the Beaumont region; and a  $0.19 \mu\text{l liter}^{-1}$  3-h average concentration in the Fort Worth region (5). These data, when compared to the significantly lower  $\text{SO}_2$  concentrations recorded in previous years (3,4), demonstrates a strong trend of increasing  $\text{SO}_2$  levels in the populated areas of Texas.

Diurnal trends of  $\text{SO}_2$  levels in metropolitan areas generally reach a maximum between 8 and 10 AM and then again during the late afternoon, with substantial drops at night (43). The  $\text{SO}_2$  concentrations and durations used in this study (chronic  $\text{SO}_2$  exposure) will probably be realized in the field (where St. Augustinegrass is extensively grown) within the next 10 years if the current trend is maintained. These considerations were the basis for the selection of concentrations and durations used.

#### Canopy Vertical Growth Rate

Because the plants were not cut during the chronic  $\text{SO}_2$  exposure period, the vertical growth rate of the canopy decreased with time. In fact, the rate of vertical elongation had decreased 90% by the second week of the chronic exposure period. This response is a well documented phenomenon (6).

While the vertical growth rate decreased for all four cultivars during the chronic  $\text{SO}_2$  fumigation period, there were no significant differences between  $\text{SO}_2$  fumigated and control plants within a

cultivar (Table I). Of the four cultivars studied, the canopy vertical growth rate was greatest for Floratam.

#### Internode Elongation

Extravaginal, aboveground, secondary lateral shoot (stolon) growth was not affected by the 5-week exposure to  $0.20 \mu\text{l liter}^{-1}$   $\text{SO}_2$ . Internode length was not different for the  $\text{SO}_2$  fumigated (+  $\text{SO}_2$ ) or control (-  $\text{SO}_2$ ) plants for any of the four cultivars (Table II). Though there were almost twice the internodes observed for the Raleigh control plants as for the fumigated plants (Table II), the values were not found to be statistically different (10% level), assuming a t distribution. Under the conditions of this study, Seville produced the greatest amount of stolon growth, while Floratam generated the least.

#### Leaf Blade Chl Content

At the end of the chronic  $\text{SO}_2$  exposure period, the Chl content of leaf blade tissue was not found to be statistically different between the fumigated or control plants for any of the four cultivars studied (Table III). The difference in mean Chl content of the  $\text{SO}_2$  fumigated and control plants can be accounted for by variability within the plant populations used.

While it has often been reported that  $\text{SO}_2$  has a detrimental affect on leaf Chl content, the reported decrease in Chl is generally accompanied by visible chlorosis, lesions, and/or necrosis (7,19,24,25,27,32). Knudson et al., (22) reported no loss of Chl in

Table I. Effect of a 5-week exposure to  $0.20 \mu\text{l liter}^{-1} \text{SO}_2$  ( $4 \text{ h day}^{-1}$ ,  $5 \text{ days week}^{-1}$ ) on the mean canopy vertical growth rate ( $\text{mm day}^{-1}$ ) of four St. Augustinegrass cultivars ( $\pm \text{SD}$ ).

Week of $\text{SO}_2$	Cultivar	$+\text{SO}_2$	$-\text{SO}_2$
First	Floritam	$20.3 \pm 2.5^1$	$19.1 \pm 2.9$
	Raleigh	$14.7 \pm 1.5$	$15.1 \pm 2.6$
	Seville	$16.0 \pm 2.8$	$14.4 \pm 1.3$
	Texas Common	$14.6 \pm 1.7$	$14.7 \pm 0.8$
Second	Floritam	$0.71 \pm 0.43$	$0.73 \pm 0.17$
	Raleigh	$0.79 \pm 0.35$	$0.81 \pm 0.38$
	Seville	$0.50 \pm 0.28$	$0.46 \pm 0.40$
	Texas Common	$0.39 \pm 0.38$	$0.43 \pm 0.31$
Third	Floritam	$0.68 \pm 0.32$	$0.70 \pm 0.11$
	Raleigh	$0.61 \pm 0.40$	$0.63 \pm 0.38$
	Seville	$0.51 \pm 0.26$	$0.48 \pm 0.21$
	Texas Common	$0.36 \pm 0.31$	$0.40 \pm 0.37$
Fourth	Floritam	$0.68 \pm 0.21$	$0.66 \pm 0.28$
	Raleigh	$0.65 \pm 0.27$	$0.61 \pm 0.10$
	Seville	$0.51 \pm 0.11$	$0.53 \pm 0.35$
	Texas Common	$0.35 \pm 0.30$	$0.40 \pm 0.42$
Fifth	Floritam	$0.60 \pm 0.31$	$0.58 \pm 0.41$
	Raleigh	$0.60 \pm 0.19$	$0.51 \pm 0.33$
	Seville	$0.53 \pm 0.27$	$0.49 \pm 0.19$
	Texas Common	$0.39 \pm 0.25$	$0.38 \pm 0.20$

<sup>1</sup> Canopy vertical growth rate ( $\text{mm day}^{-1}$ ) was calculated by dividing the change in canopy height during the week (from Saturday to Saturday) by 7 days.

Table II. Effects of a 5-week (4 h day<sup>-1</sup>, 5 days week<sup>-1</sup>) exposure to 0.20  $\mu$ l liter<sup>-1</sup> SO<sub>2</sub> on stolon internode elongation (mm) of four St. Augustinegrass cultivars ( $\pm$ SD).

Cultivar	Mean internode length		Internodes pot <sup>-1</sup>	
	- SO <sub>2</sub>	+ SO <sub>2</sub>	- SO <sub>2</sub>	+ SO <sub>2</sub>
Floritam	48 $\pm$ 11	51 $\pm$ 13	1.2	1.6 NS <sup>1</sup>
Raleigh	57 $\pm$ 17	56 $\pm$ 20	4.8	2.8 NS
Seville	47 $\pm$ 15	45 $\pm$ 13	15.8	11.2 NS
Texas Common	40 $\pm$ 12	48 $\pm$ 14	8.8	9.6 NS

<sup>1</sup> NS-not significantly different assuming a t distribution.

Table III. Effects of a 5-week (4 h day<sup>-1</sup>, 5 days week<sup>-1</sup>) exposure to 0.20  $\mu\text{l liter}^{-1}$  SO<sub>2</sub> on the mean Chl content ( $\mu\text{g mg}^{-1}$  dry weight) of the leaf blades of four St. Augustinegrass cultivars ( $\pm$  SD).

Cultivar	Charcoal-filtered air		0.20 $\mu\text{l liter}^{-1}$ SO <sub>2</sub>		$\Delta$ Chl <sup>1</sup>	
	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>
Floritam	7.01 $\pm$ 0.61	5.59 $\pm$ 0.43	6.82 $\pm$ 0.28	6.04 $\pm$ 0.49	- 3 %	+ 8 %
Raleigh	7.85 $\pm$ 0.07	6.93 $\pm$ 0.33	7.95 $\pm$ 0.86	6.84 $\pm$ 0.56	+ 1 %	- 1 %
Seville	7.52 $\pm$ 0.54	6.42 $\pm$ 0.59	7.91 $\pm$ 0.70	6.34 $\pm$ 0.47	+ 5 %	- 1 %
Texas Common	8.41 $\pm$ 0.41	5.59 $\pm$ 0.55	9.10 $\pm$ 0.93	6.04 $\pm$ 0.45	+ 8 %	+ 8 %

<sup>1</sup> The difference of the means is shown.

the leaves of Pinto bean (Phaseolus vulgaris) plants following fumigation with ozone unless injury was visibly evident. From these reports it can be concluded that a decrease in leaf Chl content following  $\text{SO}_2$  fumigation is evident only when visible injury is present, and is a consequence of extensive tissue disorder.

#### Dry Weight Accumulation

The dry weight accumulation (leaf blade plus sheath) data are presented in Table IV. As shown, the  $\text{SO}_2$  exposures statistically reduced (at the 5% level) the dry weight accumulation of the Floratam cultivar. None of the other three cultivars investigated showed a significant difference between the control and fumigated plants. Thus, it appears that the growth (photosynthesis ?) of Floratam is inhibited by low-level  $\text{SO}_2$  exposure without the appearance of visible injury (no injury or accelerated senescence was evident at the conclusion of the 5-week chronic  $\text{SO}_2$  exposure period for any of the four cultivars) or a reduction in Chl content.

It is known that sulfite, a product of  $\text{SO}_2$ , can compete with  $\text{CO}_2$  for active sites on the ribulose-1,5-bisphosphate carboxylase enzyme (19,25,41,43). The  $\text{SO}_3^{2-}$  ion would seem to be the primary sulfite species involved in photosynthetic inhibition since the pH of the chloroplast stroma is thought to be about 7.8, and optimum  $\text{CO}_2$  fixation in isolated chloroplasts has been reported to occur in a buffer solution of pH 7.6 (43). Ziegler found that "higher" concentrations of  $\text{SO}_3^{2-}$  resulted in non-competitive inhibition of the enzyme. From this, it has been suggested (25) that plants

Table IV. Effects of a 5-week (4 h day<sup>-1</sup>, 5 days week<sup>-1</sup>) exposure to 0.20  $\mu\text{l liter}^{-1}$  SO<sub>2</sub> on the mean leaf blade and sheath dry weight accumulation (g pot<sup>-1</sup>) of four St. Augustinegrass cultivars ( $\pm$  SD).

Cultivar	Dry weight		$\Delta$ dry weight <sup>1</sup>
	Charcoal-filtered air	0.20 $\mu\text{l liter}^{-1}$ SO <sub>2</sub>	
Floritam	4.20 $\pm$ 0.38	3.55 $\pm$ 0.37	-15 % (0.05)
Raleigh	3.26 $\pm$ 0.45	3.28 $\pm$ 0.74	+ 1 % NS
Seville	3.29 $\pm$ 0.26	3.48 $\pm$ 0.51	+ 6 % NS
Texas Common	3.27 $\pm$ 0.36	3.16 $\pm$ 0.64	- 3 % NS

<sup>1</sup> The difference of the means is shown. Values in parentheses show the level of significance assuming a t distribution. NS-not significant.

fixing carbon via the  $C_4$ -pathway (that is, those with a bundle sheath) would have a selective advantage over  $C_3$  plants because of an increased supply of  $CO_2$  in the bundle sheaths. In fact, decreased sensitivity to  $SO_3^{2-}$  injury has been attained by increasing  $CO_2$  concentrations (42).

If inhibition of  $CO_2$  fixation was responsible for the decreased rate of growth (dry matter accumulation) of Floratam, it appears that the other three cultivars investigated are not as readily affected. This could be due to several factors, such as, a decreased uptake of  $SO_2$  by the more "resistant" cultivars (12), higher concentrations of  $CO_2$  within the resistant plants (42), or differential  $K_i$  values of  $SO_3^{2-}$  based on unknown enzyme interactions within the different cultivars.

#### Uptake of $SO_2$

More  $SO_2$  was needed to maintain a given concentration within the fumigation chamber when the chamber contained plants. When a level of  $0.20 \mu\text{l liter}^{-1} SO_2$  was established in the chamber void of plants, and then 5 replicates of each cultivar (20 pots) were placed in the chamber, about 25 to 30 percent more  $SO_2$  had to be added to the air entering the chamber to maintain the same  $SO_2$  concentration. Although it was not verified by chemical analysis, it appears that up to 20 percent of the  $SO_2$  entering the fumigation chamber was absorbed into or onto the plants. This seems likely in light of the fact that atmospheric  $SO_2$ , in the  $\mu\text{l liter}^{-1}$  concentration range, dissolves "completely upon contact with surface or tissue

moisture of plants" (25).

Because  $\text{SO}_2$  is a gas, it is likely that the major sites of entry into a plant are the stomatal complexes. Therefore, any environmental or physiological factor affecting stomatal aperture will probably affect  $\text{SO}_2$  uptake. Although it was not controlled, the humidity within the growth chambers was consistently found to be in excess of 70% (RH) during the day (as determined by the wet-bulb, dry-bulb method). In addition, all the plants were well-watered 30 min prior to the onset of the  $\text{SO}_2$  fumigations. When these factors are coupled with the fact that all the plants were in the light for 2 h before the initiation of the  $\text{SO}_2$  exposure it seems likely that the stomatal complexes were not limiting the uptake of  $\text{SO}_2$  by the four St. Augustinegrass cultivars. Therefore, cultivar differentials realized in this study, such as the reduced growth of Floratam, should be attributed to inherent genotypic discrepancies, and not environmental parameters.

A further point to be considered is the affect of closed stomatal complexes on plant growth. If the three unaffected cultivars, Raleigh, Seville, and Texas Common, were protected from  $\text{SO}_2$ -induced growth inhibition via closure of the stomatal complexes during the repeated  $\text{SO}_2$  challenges, the resulting limitations on  $\text{CO}_2$  exchange would have inhibited growth (dry matter accumulation). However, this was not the case.

#### Acute $\text{SO}_2$ Exposure

#### Visible Injury

Twenty hours after the termination of the acute (1.0  $\mu\text{l liter}^{-1}$ , 10 h  $\text{day}^{-1}$ , for 4 consecutive days)  $\text{SO}_2$  exposure period, the three potted turfs of each cultivar used during the fumigations were visually rated for injury (0-no injury, 9-severe injury). An analysis of the mean ratings by Duncan's new multiple range test showed that Floratam was significantly (5% level) more injured than Texas Common, which was significantly (5% level) more injured than either Raleigh or Seville, which were injured to the same degree. There were visually apparent cultivar differentials within the St. Augustinegrass species.

Symptoms of injury to the leaves of St. Augustinegrass plants was typical of injury to other grasses, as reported in the literature (10,27,28,31,33,35). The appearance and progression of injury to St. Augustinegrass was as follows: During the middle of the fourth day of acute  $\text{SO}_2$  exposure, small areas with a water-soaked appearance were evident on some leaves of the Floratam plants. By the end of the fourth day, some of these areas had turned a dull gray or brown, with symptoms also observed on the leaves of Texas Common. Later, (within 20 h) the affected areas had turned purple, or whitish-purple, with bifacial collapse clearly evident.

Injury was generally restricted to the terminal ends of old as well as younger leaves. No preferential injury based on leaf age was observed. Injured tissue at times exhibited a banding pattern across the leaf blades, with the lesions following the pattern of the leaf veins. Some injury was observed at the base of the leaf

blades and even along the margins of the leaf sheathes.

Twenty h after the termination of the acute  $\text{SO}_2$  fumigation period, leaves of the two most affected cultivars, Floratam and Texas Common, were excised and photographed (Figs. 3 and 4).

Plants categorized as  $\text{SO}_2$ -sensitive are severely injured by a few hours (<5) of  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$ , while moderately sensitive plants are visibly injured by less than 10 h of  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$  (35). Since 4 days ( $10 \text{ h day}^{-1}$ ) were required for the induction of visible injury of St. Augustinegrass by  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$ , the St. Augustinegrass species should be classified as resistant to  $\text{SO}_2$ -induced injury. Since these classifications (see refs. 10 and 35 for a description of classification criteria) are based on acute exposures, which are not necessarily correlated to chronic  $\text{SO}_2$ -induced injury (20), these data alone do not indicate that St. Augustinegrass is not affected by  $\text{SO}_2$  in the field. In fact, the data accumulated during the Chronic  $\text{SO}_2$  exposure portion of these investigations indicate that at least one cultivar (Floratam) is detrimentally affected by low-level  $\text{SO}_2$ .

#### Chl Content of Leaf Blades

The loss of leaf blade Chl (control mean content minus fumigated mean content) as a result of the acute  $\text{SO}_2$  fumigations was calculated. No significant differences were found between the control and  $\text{SO}_2$ -fumigated plants for any of the four cultivars (data not shown). Since no more than 5% of the total leaf blade area of an entire pot was visibly injured, the loss of Chl within

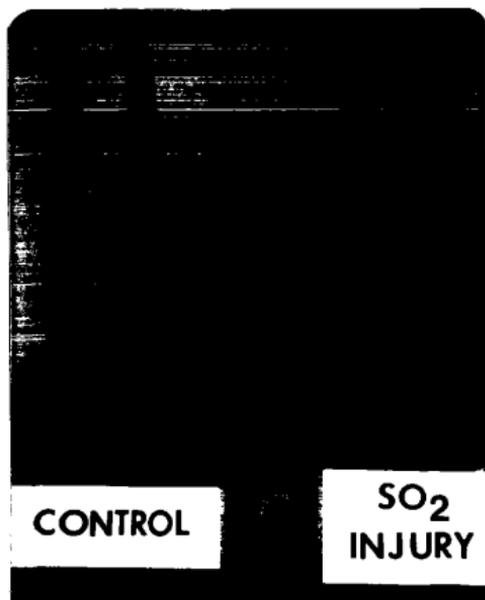


Fig. 3. Symptoms of  $\text{SO}_2$ -induced injury to Floratam St. Augustinegrass. The leaves on the right are from plants that were exposed to  $1.0 \mu\text{l liter}^{-1}$   $\text{SO}_2$  for four consecutive days ( $10 \text{ h day}^{-1}$ ). This photograph was taken 20 h after termination of the  $\text{SO}_2$  fumigation on the fourth day.

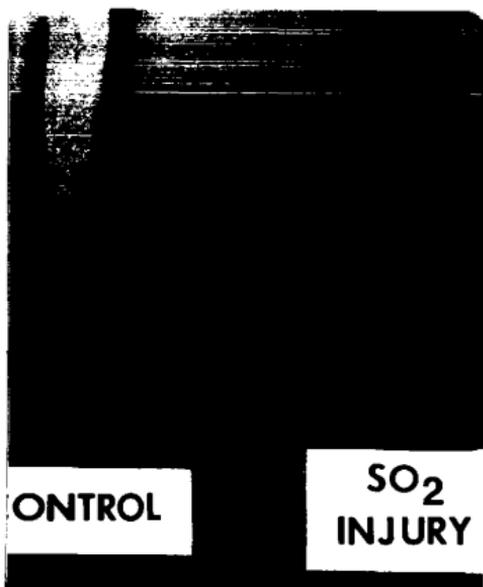


Fig. 4. Symptoms of  $\text{SO}_2$ -induced injury to Texas Common St. Augustinegrass. The leaves on the right are from plants that were exposed to  $1.0 \mu\text{l liter}^{-1}$   $\text{SO}_2$  for four consecutive days ( $10 \text{ h day}^{-1}$ ). This photograph was taken 20 h after termination of the  $\text{SO}_2$  fumigation on the fourth day.

the injury lesions would not result in an appreciable change in total Chl, at least not on a total dry weight basis. Again, as Knudson *et al.* (22) showed, when 30% of the leaf area of Pinto beans were injured (visibly) by ozone, the accompanying loss of leaf blade Chl was <30%, on a dry weight basis. So it seems, Chl content of leaf tissue can only be used as a quantitative measure of air pollution injury if the leaf tissue is "severely injured".

#### Bisulfite Ion Treatment

It is much more convenient to float leaf tissue on a solution containing  $\text{HSO}_3^-$  than to fumigate whole plants with  $\text{SO}_2$ . This is particularly true in two instances. When determining the  $\text{SO}_2$  sensitivity of a large number of plants, and when limited plant material is available, such as germplasm sources. Ideally, tissue from field grown plants can be exposed to  $\text{HSO}_3^-$  containing solutions without the stabilization time required when moving whole plants from the field to a growth chamber. Air samples (for hydrocarbon analysis) can be easily obtained and chromatographed since no sample preparation is necessary. Also, the destruction of plants is not entailed as only a few leaves are obligatory for the assay.

#### Ethylene and Ethane Production

Although there was a 15 to 30 minute period realized for the accumulation of the produced gases before the first gas sampling (0 h values for Figs. 5 to 20) and subsequent chromatography, the 0 h values for ethylene and ethane do,

in general, represent ambient levels of the two gases at the time the flasks were sealed. Any ethylene or ethane being generated by the flask stoppers etc., would have been accounted for by the controls ( $K_2SO_4$ ). Likewise, the ethylene, and to a lesser degree ethane, being produced as a consequence of the leaf tissue being injured by cutting and then exposure to an acidic solution would have been expressed in the controls as well as the  $KHSO_3$  treated tissue.

Since the level of ambient ethylene and ethane varied from experiment to experiment, the rate of production (slope of the concentration vs. time line) of the two gases would be a good measure of the hydrocarbon production. The ethylene and ethane being produced as a result of the  $KHSO_3$  ( $HSO_3^-$ ) exposure can be determined by calculating the difference of the slope for the  $KHSO_3$  and  $K_2SO_4$  (control) treated tissue.

The rates of hydrocarbon production were calculated by utilizing the following equation:

$$R = \frac{E_{48} - E_0}{48 \text{ h}} \quad (7)$$

where  $R$  is the rate of production in  $nl \text{ mm}^{-2} \text{ h}^{-1}$ ,  $E_{48}$  is the ethylene or ethane accumulated in the flask 48 h after the exposure,  $E_0$  is the ethylene or ethane present in the flask during the first (0 h) sampling, and 48 h is the change in time from the first sampling to the last. This formula does not account for non-linearity, but assumes a constant rate of production for 48 h.

Bressan et al. (11) exposed leaf discs from 35 cultivars of the Cucurbitaceae family to 50 mM  $\text{KHSO}_3$  solutions for 2 h and measured ethane accumulation after 24 h. After modifying equation (7) by replacing  $E_{48}$  with  $E_{24}$  and 48 h with 24 h, the author utilized the data presented by Bressan et al (11) to determine the slope of the ethane production line for the most and least injured cultivars. The two least injured by the  $\text{KHSO}_3$  exposure, as well as by an  $\text{SO}_2$  fumigation, (Citrullus lanatus plant introductions 246029 and 192937) showed ethane production rates of  $2.50 \text{ nl mm}^{-2} \text{ h}^{-1}$  (treated minus control). In contrast, the most injured cultivar, Cucurbita mixta cv. Cinderella, showed an ethane production rate of  $107 \text{ nl mm}^{-2} \text{ h}^{-1}$ . For comparison, the four cultivars of St. Augustinegrass which were exposed to  $\text{KHSO}_3$  (50 mM) for 2 h in this study, exhibited ethane production rates of 1.42, 1.42, 1.63, and 1.96  $\text{nl mm}^{-2} \text{ h}^{-1}$  (treated minus control) for the 24 h period immediately following the  $\text{KHSO}_3$  exposure (Seville, Floratam, Raleigh, and Texas Common, respectively). This comparison indicates that these cultivars (St. Augustinegrass) are resistant to  $\text{SO}_2$ -induced injury, which was indeed found to be the case during the acute exposure portion of these investigations. It should be noted that exposure to bisulfite is a test of a plants ability to tolerate acute, not chronic, exposures to  $\text{SO}_2$ .

Since the  $\text{K}_2\text{SO}_4$  treated (control) tissue generally emitted as much ethylene as the tissue exposed to  $\text{KHSO}_3$  (Figs. 5 to 12), it seems reasonable to assume that ethylene was being produced

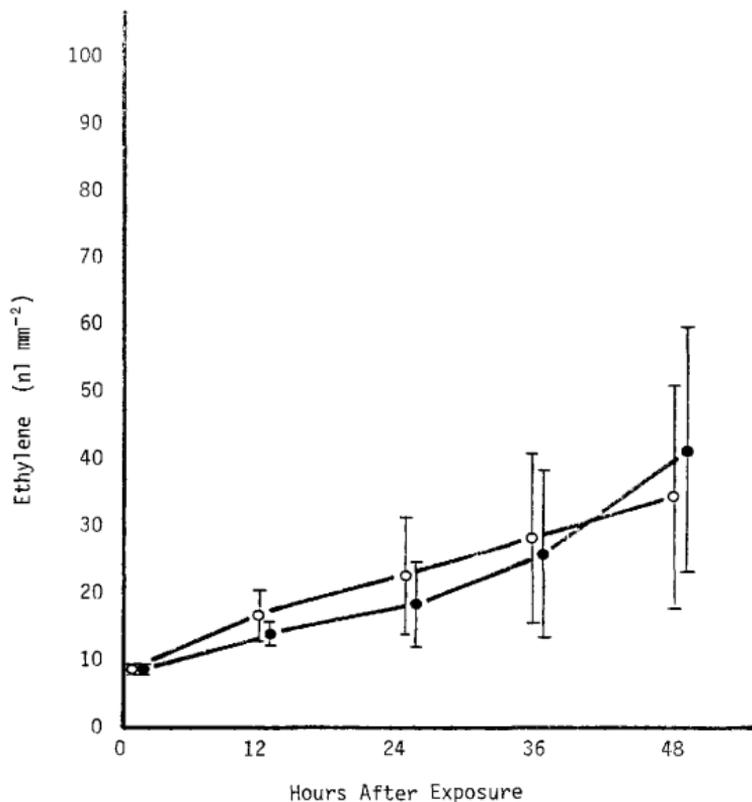


Fig. 5. Mean ethylene production ( $\text{nl mm}^{-2}$ ) by leaf sections of Floratam St. Augustinegrass following a 2 h exposure to 10 mM  $\text{KHSO}_3$  (●) or 10 mM  $\text{K}_2\text{SO}_4$  (○).  $\text{K}_2\text{SO}_4$  was used as a control. Vertical lines show one standard deviation.

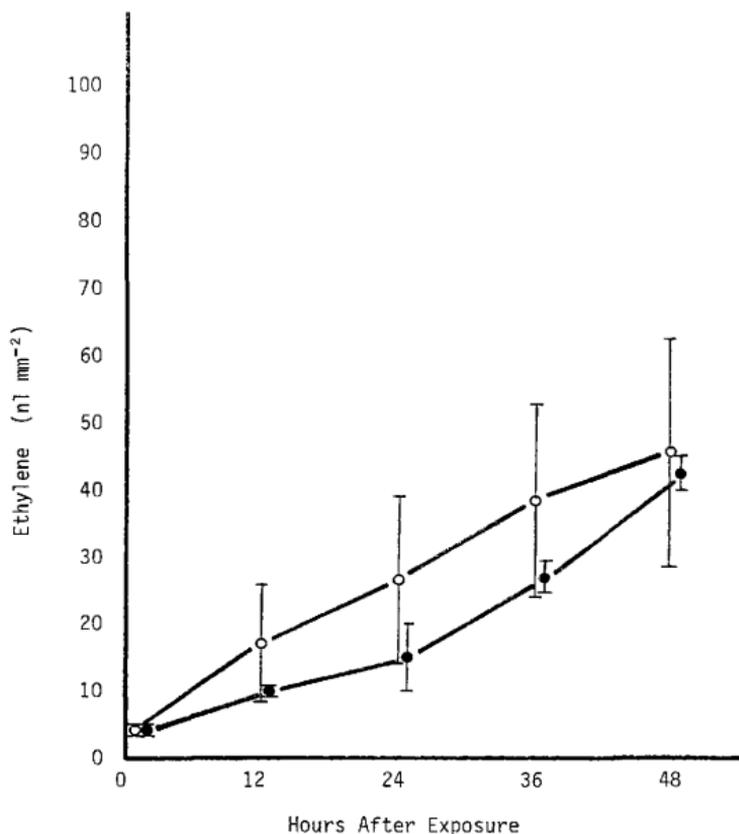


Fig. 6. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Raleigh St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

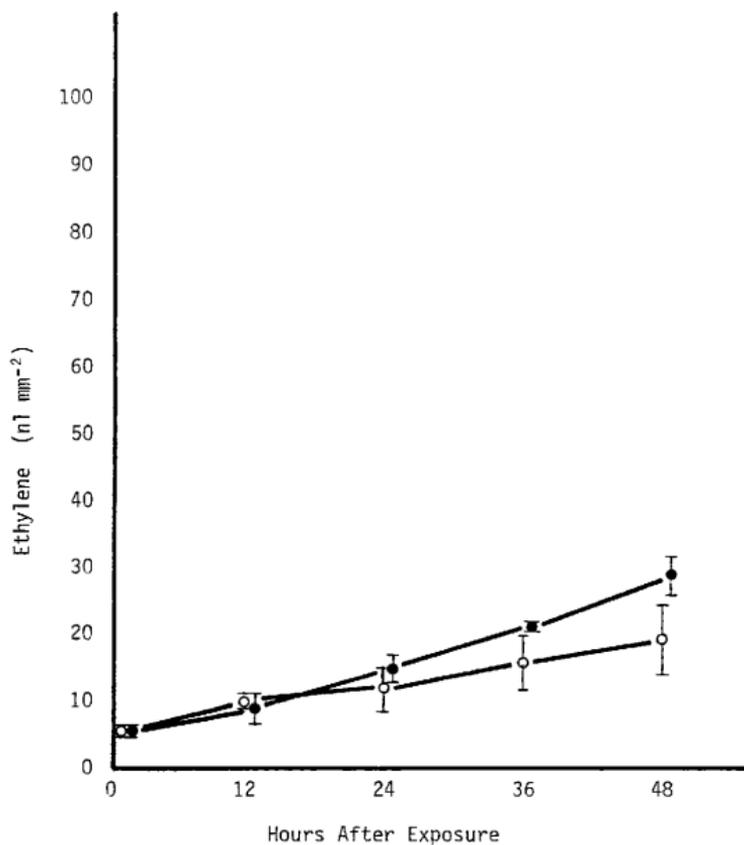


Fig. 7. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Seville St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

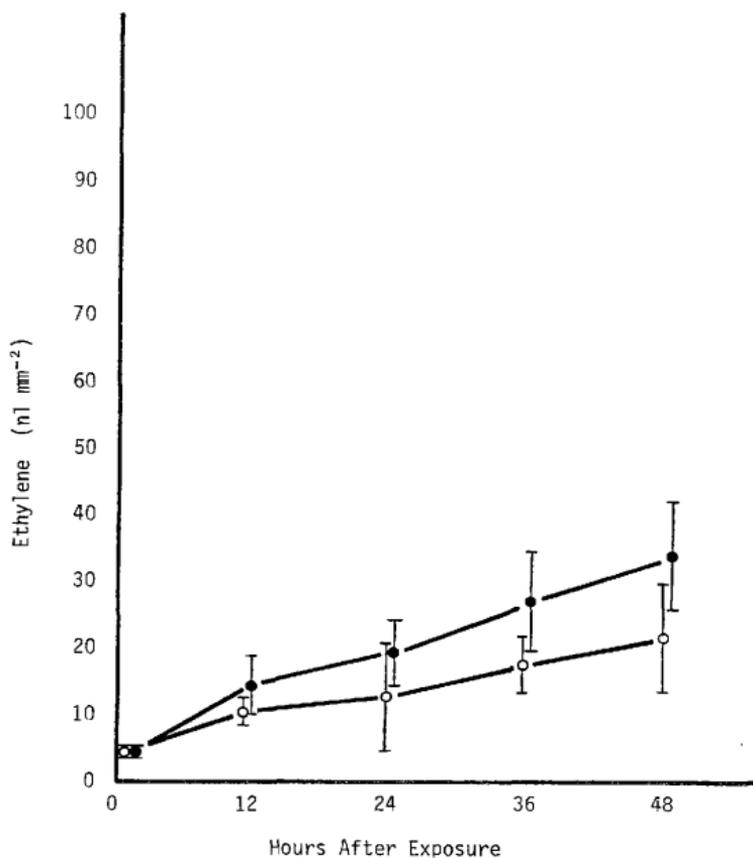


Fig. 8. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Texas Common St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

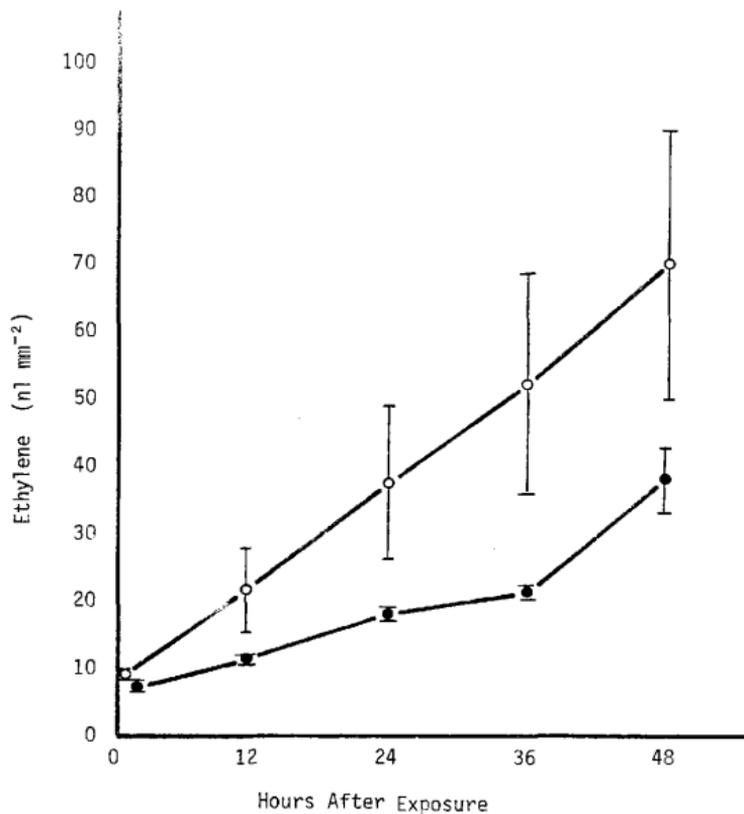


Fig. 9. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Floratam St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

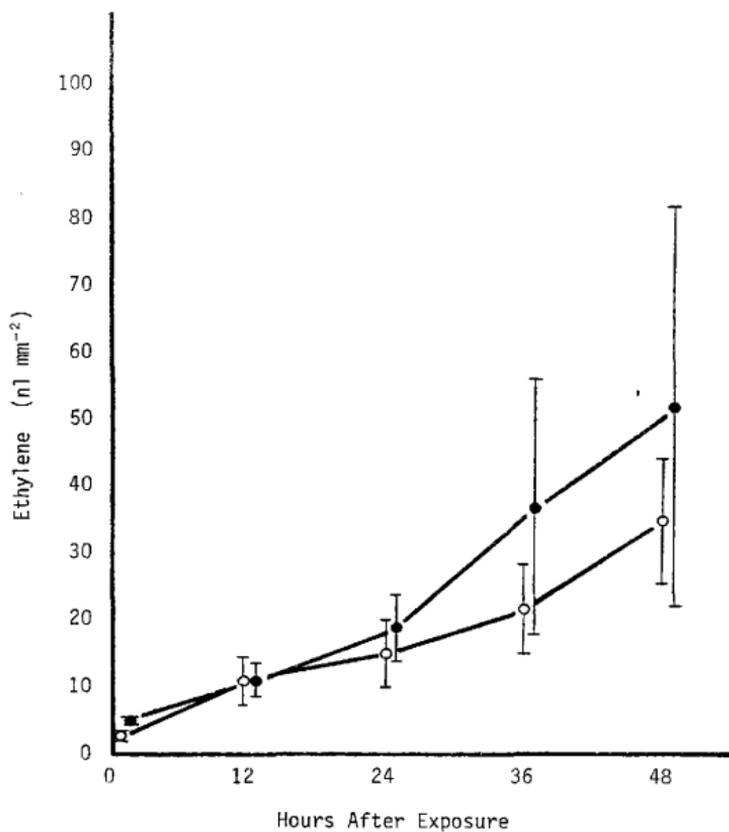


Fig. 10. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Raleigh St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

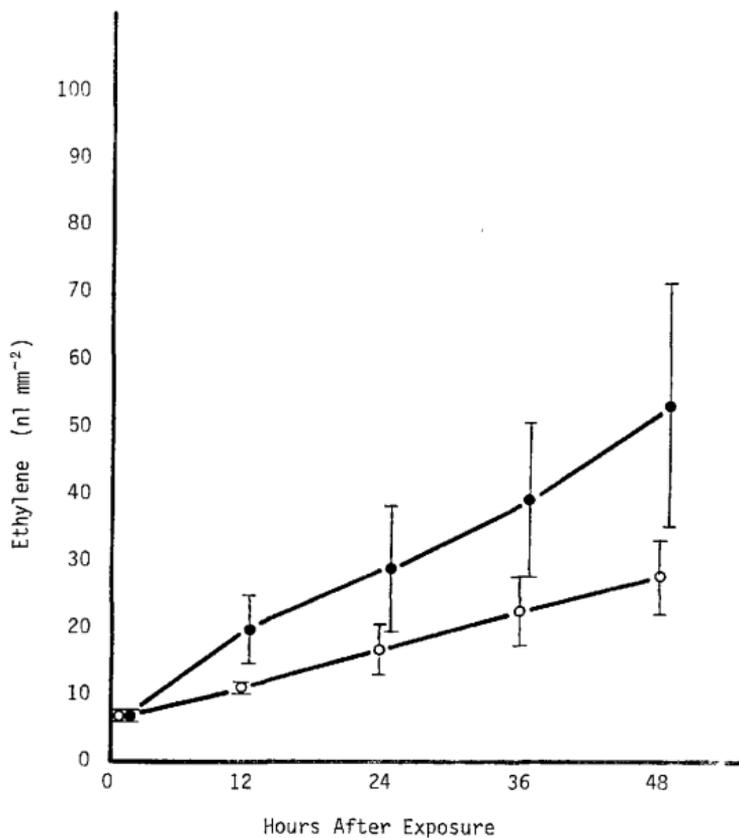


Fig. 11. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Seville St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

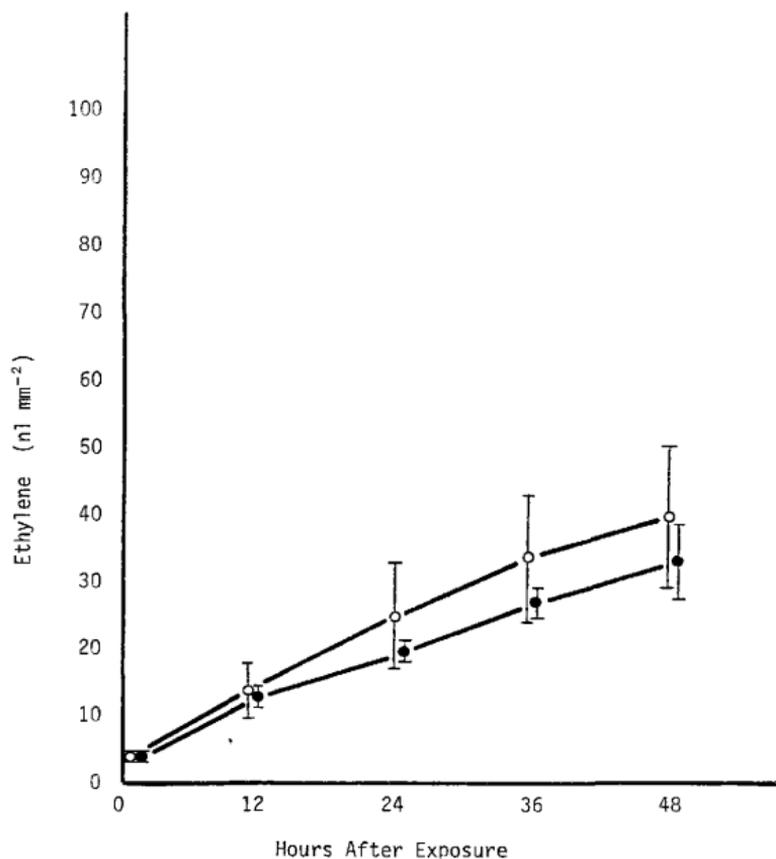


Fig. 12. Mean ethylene production (nl mm<sup>-2</sup>) by leaf sections of Texas Common St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

largely in response to the tissue being cut, and then further injured by an acidic (pH 4.2 - 4.3) solution. A notable exception can be seen in Fig. 9, where the Floratam tissue exposed to 50 mM  $K_2SO_4$  produced twice the ethylene that the leaf sections exposed to 50 mM  $KHSO_3$  did.

Ethane, on the contrary, was not produced nearly as extensively by control tissue as by tissue exposed to  $KHSO_3$  (Figs. 13 to 20). Since the difference between the two solutions was the sulfur containing ion, it seems that ethane was produced as a specific response to  $HSO_3^-$  injury. This is also the conclusion drawn by Bressan et al. (11).

The rates of ethane production as calculated by equation (7), are compared to the visible injury ratings following the acute  $SO_2$  exposure for each cultivar in Table V. The ethane assay showed Floratam and Texas Common to be more susceptible to  $SO_2$ -induced injury than either Raleigh or Seville, but did not predict the difference in susceptibility (based on visual ratings) between Floratam and Texas Common. Thus, the ethane assay (48 h, not 24 h) was at least moderately sensitive in predicting cultivar discrepancy within the St. Augustinegrass species.

It is of course possible that the ethane assay would be much more sensitive with more susceptible (to  $SO_2$ -induced injury) species and cultivars, than was found for the plant material used in these investigations. Certainly, Bressan et al. (11) maintain this view. The validity of the visual ratings

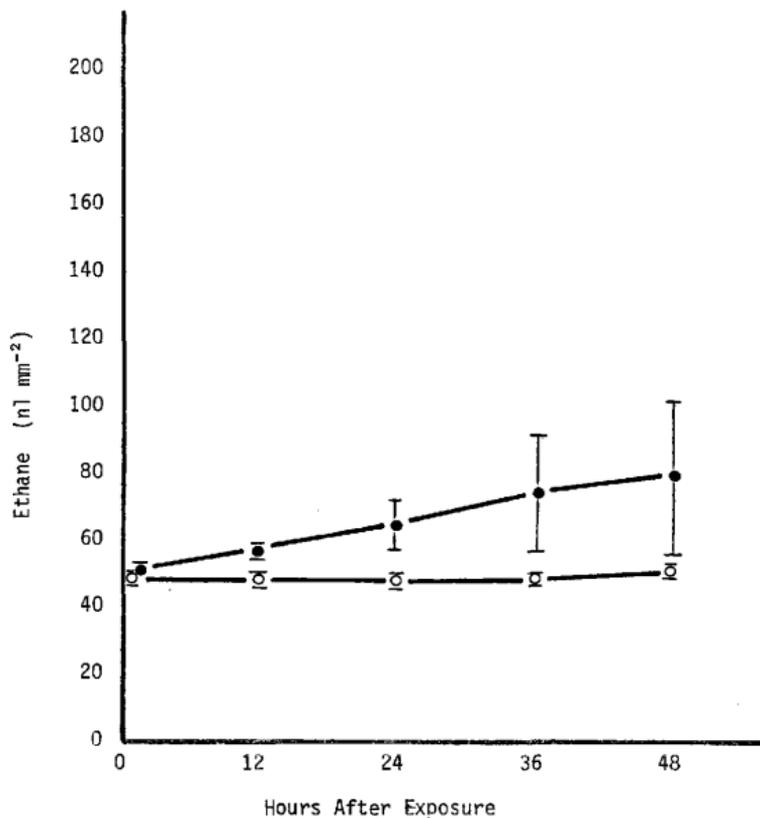


Fig. 13. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Floratam St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

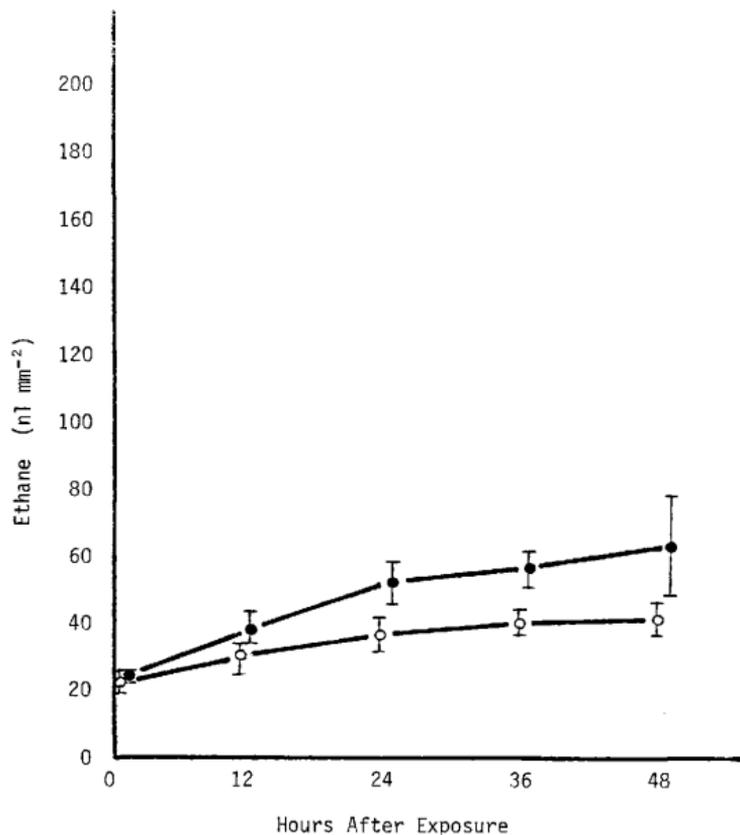


Fig. 14. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Raleigh St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

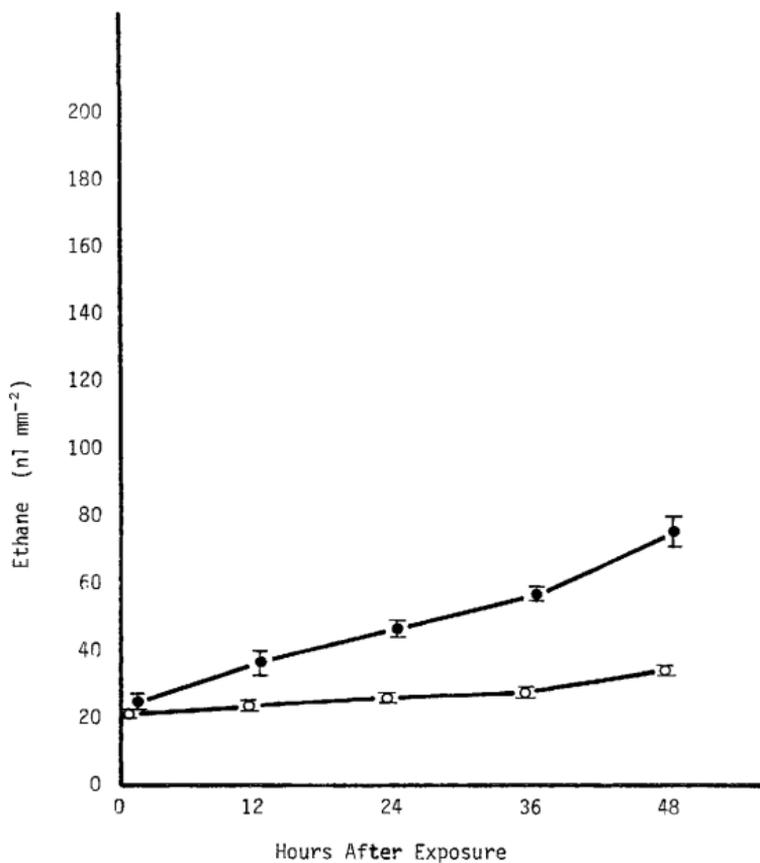


Fig. 15. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Seville St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

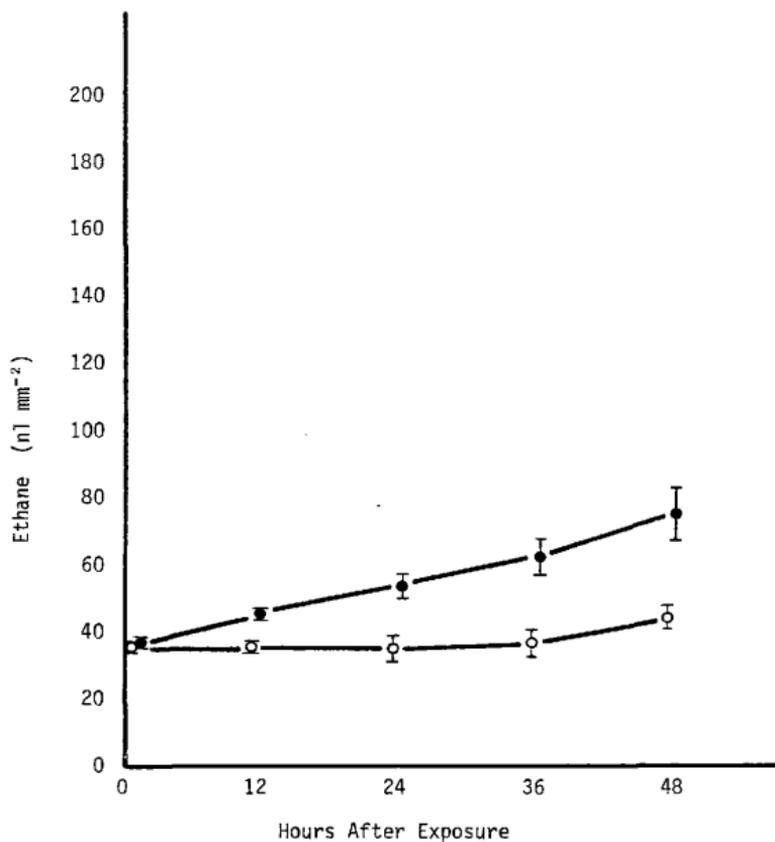


Fig. 16. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Texas Common St. Augustinegrass following a 2 h exposure to 10 mM KHSO<sub>3</sub> (●) or 10 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

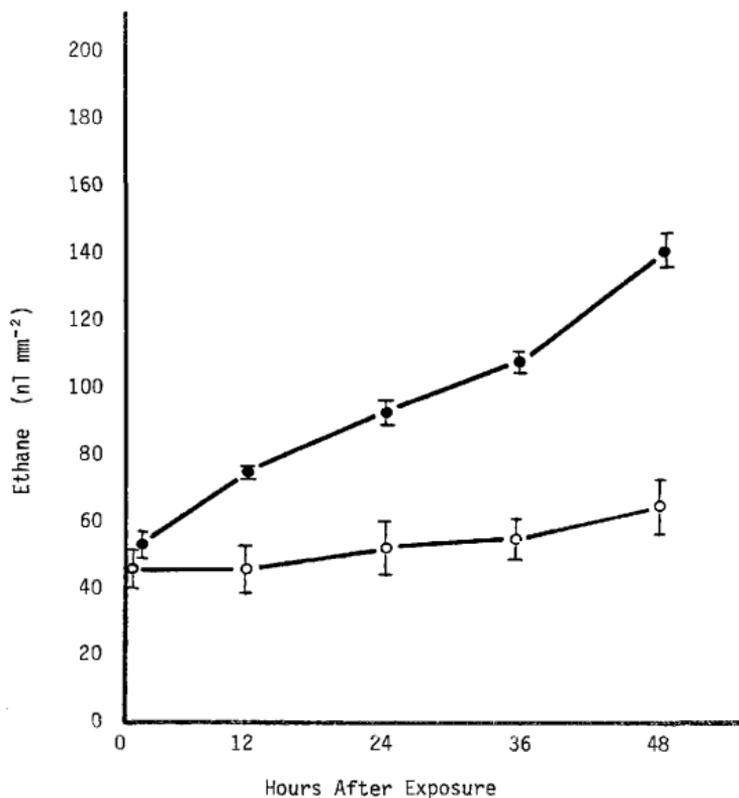


Fig. 17. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Floratam St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

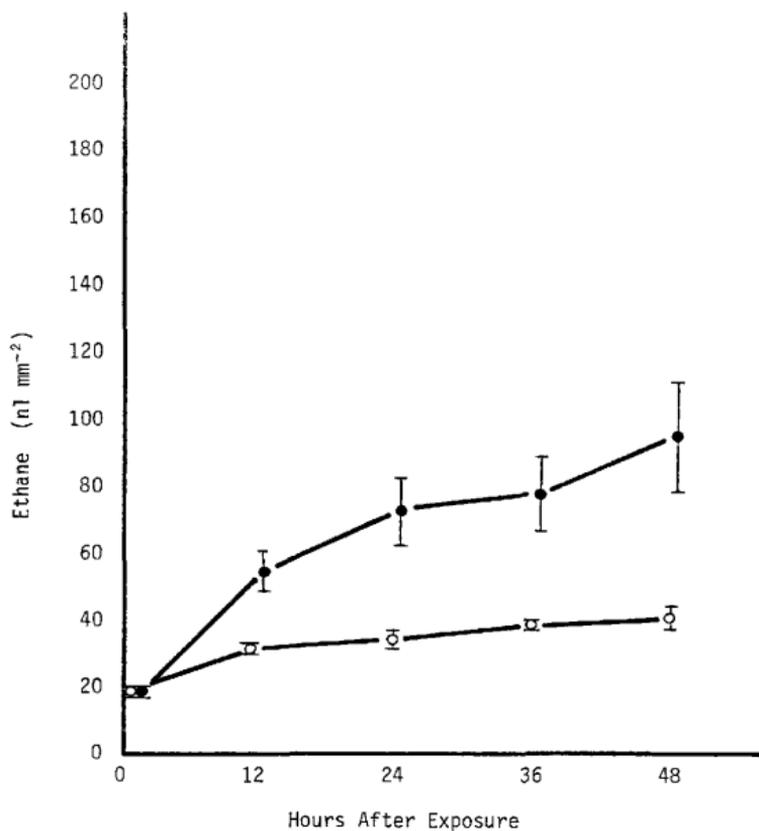


Fig. 18. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Raleigh St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

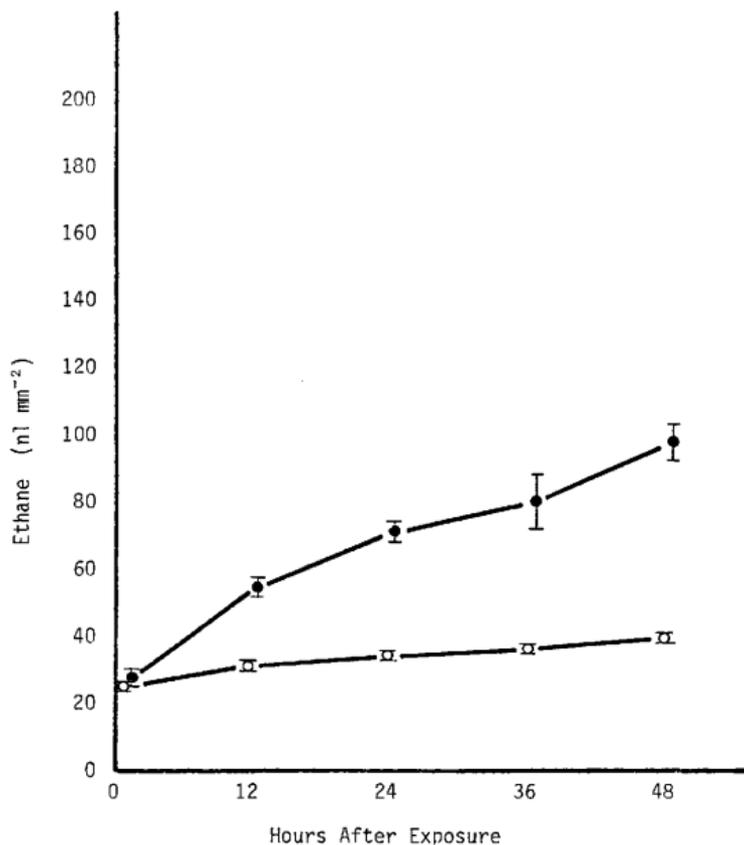


Fig. 19. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Seville St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

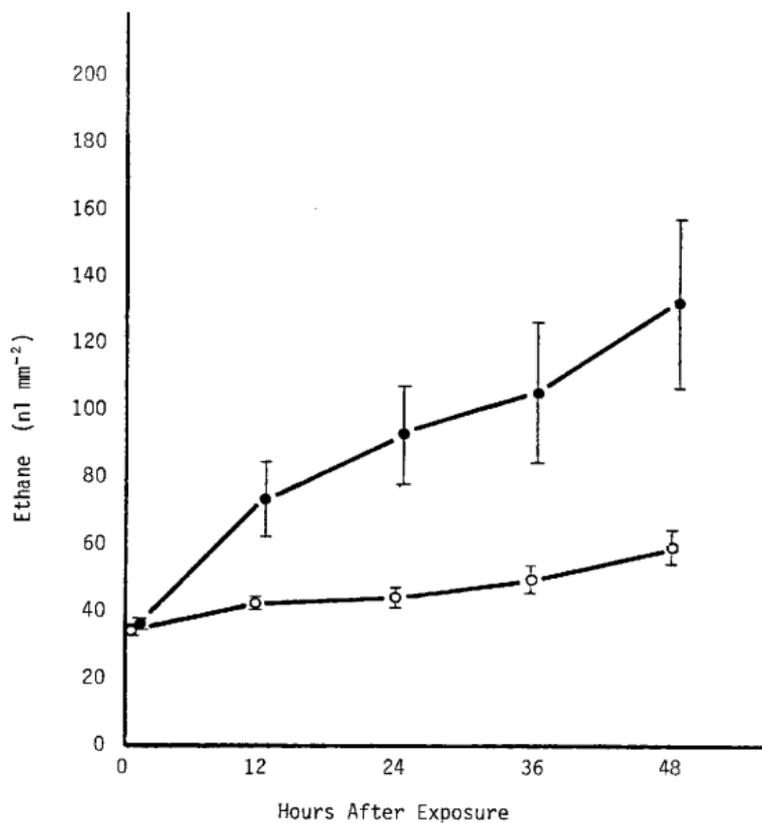


Fig. 20. Mean ethane production (nl mm<sup>-2</sup>) by leaf sections of Texas Common St. Augustinegrass following a 2 h exposure to 50 mM KHSO<sub>3</sub> (●) or 50 mM K<sub>2</sub>SO<sub>4</sub> (○). K<sub>2</sub>SO<sub>4</sub> was used as a control. Vertical lines show one standard deviation.

Table V. Comparison of the rate of ethane production for 48 h following a 2 h exposure to 50 mM  $\text{KHSO}_3$ , and mean visible injury ratings 20 h after fumigation with  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$  (10 h  $\text{day}^{-1}$ , 4 consecutive days) of four St. Augustinegrass cultivars.

Cultivar	Rate of ethane production <sup>1</sup> ( $\text{nl mm}^{-2} \text{h}^{-1}$ )	Visible injury rating <sup>2</sup>
Floritam	1.48	2.7 a <sup>3</sup>
Texas Common	1.48	1.0 b
Raleigh	1.15	0.3 c
Seville	1.15	0.2 c

<sup>1</sup> Rate of ethane production was determined using equation (7) (see text) for both the  $\text{KHSO}_3$  and  $\text{K}_2\text{SO}_4$  treatments. The rate of ethane production as a result of  $\text{KHSO}_3$  injury was assumed to be the difference of the two treatment rates.

<sup>2</sup> Visual ratings 20 h after the termination of the 4-day  $\text{SO}_2$  fumigation period (see text for details). 0-no injury, 9-severe injury.

<sup>3</sup> Means followed by the same letter are not significantly different (5% level) according to Duncan's new multiple range test.

(following the acute exposures to  $\text{SO}_2$ ) must also be taken into account when evaluating the sensitivity of the ethane assay. In this case, at least, the cultivar differentials following the acute  $\text{SO}_2$  exposures were quite clear.

Data from the analysis of ethane production correctly predicted two St. Augustinegrass- $\text{SO}_2$  interactions: the species is quite resistant to  $\text{SO}_2$ -induced injury, and Seville and Raleigh are the two most resistant cultivars, of the four investigated.

#### Visible Injury and Chl Content

As shown in Tables VI and VII, the visible injury sustained by all four cultivars following exposure to either 10 or 50 mM  $\text{KHSO}_3$  was similar, with the tissue exposed to the 10 mM  $\text{KHSO}_3$  solution injured 20 to 30 percent, and the tissue exposed to the 50 mM  $\text{KHSO}_3$  solution injured about 50 to 60 percent (see the Table footnotes for the evaluation technique employed). While the visible injury did not differ significantly among the four cultivars, the Chl content following the exposures did.

As Table VIII shows, Floratam sustained the greatest loss in Chl as a consequence of exposure to 10 mM  $\text{KHSO}_3$ , with Seville being least affected. Similarly, Floratam and Texas Common showed the largest loss in Chl following exposure to 50 mM  $\text{KHSO}_3$ , with Seville being least affected (Table IX). These data suggest that Floratam and Texas Common would be injured to a greater extent by an acute  $\text{SO}_2$  exposure than would Seville. This was found to be true in the acute exposure portion of these investigations.

Table VI. Mean percent injury to leaf blade sections of four St. Augustinegrass cultivars 48 h after a 2 h exposure to a 10 mM solution of either  $K_2SO_4$  or  $KHSO_3$  ( $\pm$  SD).

Cultivar	% Injury <sup>1</sup>	
	$K_2SO_4$ (control)	$KHSO_3$
Floritam	<5 $\pm$ <5	21 $\pm$ 5
Raleigh	<5 $\pm$ <5	25 $\pm$ 5
Seville	<5 $\pm$ <5	24 $\pm$ 5
Texas Common	<5 $\pm$ <5	26 $\pm$ 2

<sup>1</sup> Injury was assessed by visually comparing leaf sections to drawings of leaf sections (enlarged) with various (20, 40, 60, and 80 %) areas blackened to represent necrosis.

Table VII. Mean percent injury to leaf blade sections of four St. Augustinegrass cultivars 48 h after a 2 h exposure to a 50 mM solution of either  $K_2SO_4$  or  $KHSO_3$  ( $\pm$  SD).

Cultivar	% Injury <sup>1</sup>	
	$K_2SO_4$ (control)	$KHSO_3$
Floritam	<5 $\pm$ <5	55 $\pm$ 6
Raleigh	<5 $\pm$ <5	55 $\pm$ 8
Seville	<5 $\pm$ <5	53 $\pm$ 3
Texas Common	<5 $\pm$ <5	60 $\pm$ 6

<sup>1</sup> Injury was assessed by visually comparing leaf sections to drawings of leaf sections (enlarged) with various (20, 40, 60, and 80 %) areas blackened to represent necrosis.

Table VIII. Mean Chl content ( $\mu\text{g mg}^{-1}$  dry weight) of leaf sections of four St. Augustinegrass cultivars 48 h after a 2 h exposure to a 10 mM solution of either  $\text{K}_2\text{SO}_4$  or  $\text{KHSO}_3$  ( $\pm$  SD).

Cultivar	$\text{K}_2\text{SO}_4$ (control)		$\text{KHSO}_3$		$\Delta$ Chl <sup>1</sup>	
	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>
Floritam	5.78 $\pm$ 0.57	5.96 $\pm$ 0.49	4.18 $\pm$ 0.39	4.76 $\pm$ 0.41	-28 %	-20 %
Raleigh	7.95 $\pm$ 0.51	5.90 $\pm$ 0.33	6.25 $\pm$ 0.37	5.73 $\pm$ 0.42	-21 %	- 3 %
Seville	7.09 $\pm$ 0.68	5.66 $\pm$ 0.51	6.24 $\pm$ 0.51	5.58 $\pm$ 0.29	-12 %	- 1 %
Texas Common	7.55 $\pm$ 0.71	6.02 $\pm$ 0.55	6.61 $\pm$ 0.57	5.57 $\pm$ 0.47	-12 %	- 7 %

<sup>1</sup> The difference of the means is shown.

Table IX. Mean Chl content ( $\mu\text{g mg}^{-1}$  dry weight) of leaf sections of four St. Augustinegrass cultivars 48 h after a 2 h exposure to a 50 mM solution of either  $\text{K}_2\text{SO}_4$  or  $\text{KHSO}_3$  ( $\pm$  SD).

Cultivar	$\text{K}_2\text{SO}_4$ (control)		$\text{KHSO}_3$		$\Delta$ Chl <sup>1</sup>	
	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>	Chl <u>a</u>	Chl <u>b</u>
Floritam	5.85 $\pm$ 0.45	6.35 $\pm$ 0.52	3.97 $\pm$ 0.40	5.47 $\pm$ 0.41	-32 %	-14 %
Raleigh	7.51 $\pm$ 0.53	5.77 $\pm$ 0.31	5.81 $\pm$ 0.51	4.88 $\pm$ 0.18	-23 %	-15 %
Seville	7.08 $\pm$ 0.60	5.93 $\pm$ 0.33	6.16 $\pm$ 0.47	5.44 $\pm$ 0.34	-13 %	- 8 %
Texas Common	7.87 $\pm$ 0.63	5.70 $\pm$ 0.49	5.56 $\pm$ 0.59	4.38 $\pm$ 0.39	-29 %	-23 %

<sup>1</sup> The difference of the means is shown.

Chl a was more readily destroyed than Chl b by a 2 h exposure to  $\text{KHSO}_3$  containing solutions in this study (Tables VIII and IX). This is typical of reports in the literature (24,29,30,32). Although the preferential destruction of Chl a has also been reported for both  $\text{SO}_2$  (25) and ozone (22), as well as bisulfite, no explanation of this discrepancy has been forwarded in the literature.

It must be noted that light has a marked affect on both hydrocarbon production and visible injury expression following an  $\text{SO}_2$  fumigation (31). This has also been reported to be true for an ozone fumigation (17). To eliminate any complications resulting from environmental factors, tissue being a) incubated prior to gas sampling for hydrocarbon analysis, or b) monitored for the appearance of visible injury, should be kept under a strict light-dark regime as was the case during these investigations.

#### Further Statistical Analysis

Least squares linear regression models were developed for two relationships between data sets accumulated in these investigations as follows (see previous pages for units used):

When the reduction of dry matter accumulation (y) following the 5-week exposure to  $0.20 \mu\text{l liter}^{-1} \text{SO}_2$  ( $4 \text{ h day}^{-1}$ ,  $5 \text{ days week}^{-1}$ ) was plotted against the visual injury ratings (x) following exposure to  $1.0 \mu\text{l liter}^{-1} \text{SO}_2$  for four consecutive

days (10 h day<sup>-1</sup>), the following model was developed:

$$y = -5.22 + 7.59(x) \quad r^2 = 0.960 \quad (8)$$

When the visual injury ratings ( $y$ ) following exposure to 1.0  $\mu\text{l liter}^{-1}$   $\text{SO}_2$  for four consecutive days (10 h day<sup>-1</sup>) were plotted against the loss ( $\text{K}_2\text{SO}_4$  treated minus  $\text{KHSO}_3$  treated) of total Chl ( $\underline{a} + \underline{b}$ ) ( $x$ ) following a 2 h exposure to either  $\text{K}_2\text{SO}_4$  or  $\text{KHSO}_3$  (10 mM), the following model was developed:

$$y = -0.922 + 0.144(x) \quad r^2 = 0.809 \quad (9)$$

In addition, a least squares exponential regression model was developed for the relationship between the visual injury ratings ( $y$ ) following exposure to 1.0  $\mu\text{l liter}$   $\text{SO}_2$  for four consecutive days (10 h day<sup>-1</sup>) and the 48 h ethane production rate ( $x$ ), as calculated with equation (7), following exposure to both 50 mM  $\text{KHSO}_3$  and  $\text{K}_2\text{SO}_4$  as follows:

$$y = 0.085(x)^{7.55} \quad r^2 = 0.863 \quad (10)$$

These models may be beneficial in a number of ways. One, by utilizing equation (8), short-term acute  $\text{SO}_2$  exposures can be used to predict the affects of long-term chronic exposures to  $\text{SO}_2$  on the growth of St. Augustinegrass cultivars. Two, by utilizing equation (9), a simple chemical test can be used to predict the level of resistance of a particular St. Augustinegrass cultivar to  $\text{SO}_2$ -induced injury. Three, by utilizing equation (10), the "bisulfite/ethane system" can be used to screen a large number of cultivars of St. Augustinegrass for relative resistance to injury

induced by acute exposures to  $\text{SO}_2$ .

#### Proposed Future Research

This study has established that under laboratory growth chamber conditions, St. Augustinegrass cultivars respond differently to  $\text{SO}_2$  exposures. The growth of Floratam, a major commercial cultivar, was significantly inhibited by the chronic  $\text{SO}_2$  fumigations employed during these investigations. Testing of more cultivars using a similar fumigation system would provide valuable information to turfgrass managers. Floratam, and at least one of the other cultivars used in this study, should be used as internal standards for any additional research. During such studies, several cultural factors (i.e. mowing, irrigation, pesticide application, and fertilization) could be investigated for their influence on injury expression.

Possibly the most practical and sensible test of the effects of  $\text{SO}_2$  on St. Augustinegrass would be accomplished with the use of field chambers as described by Bell and Mudd (7). With the use of such chambers, field grown plants can be exposed to controlled levels of  $\text{SO}_2$  as well as being subject to "real-world" environmental fluctuations. If field studies are conducted in  $\text{SO}_2$ -polluted areas, control (unfumigated) plants can be grown by passing air entering a field chamber through a charcoal-filter to remove the  $\text{SO}_2$ , while  $\text{SO}_2$  fumigated plants can be grown in the ambient air. This system would necessitate the continuous monitoring of ambient  $\text{SO}_2$  levels.

In short, data from these investigations indicate that St. Augustinegrass is, or could be, affected by  $\text{SO}_2$ -polluted atmospheres in the urban and industrial areas of the Southern United States. Therefore, further research into the detrimental affects of  $\text{SO}_2$  is warranted.

## SUMMARY AND CONCLUSIONS

There exists significant diversity in susceptibility to injury induced by  $\text{SO}_2$  among plant species and even among cultivars within a species. In these investigations, the growth of Floratam was inhibited by the chronic  $\text{SO}_2$  exposures, while a trend (not statistically significant, however) toward increased growth was observed for Seville. Floratam was the most injured by acute  $\text{SO}_2$  exposures of the four cultivars used, while Seville was least injured. Further research is needed to determine whether plants resistant to acute  $\text{SO}_2$  exposures (i.e. Seville in this study), are benefited by low-level exposures.

While injury induced by  $\text{SO}_2$  is generally evaluated by visual means, more subtle "invisible injury" is often realized, but not immediately recognized. In these studies, Floratam was subject to "invisible injury" when exposed to long-term, low-level  $\text{SO}_2$ . Without quantitative measurement, this subtle injury would have gone unrecognized.

Laboratory studies generally involve acute  $\text{SO}_2$  fumigations while field grown plants are most often exposed to long-term, low-level  $\text{SO}_2$ . Though acute laboratory fumigations may be of benefit when studying the mechanisms of  $\text{SO}_2$ -induced injury, and evaluating cultivars for relative injury resistance, field studies are the most legitimate means of investigating  $\text{SO}_2$ -plant interactions of practical ("real world") significance. Now that

some basic information regarding the effects of  $\text{SO}_2$  on St. Augustinegrass has been accumulated and analyzed, this should be used to design more elaborate field investigations.

The exact mechanisms of injury to plants during and after an  $\text{SO}_2$  challenge are not completely understood. Many investigators have concluded that the destruction of Chl by  $\text{SO}_2$  is a major link in the chain of events leading to plant injury. In addition, analysis of leaf Chl content is a quantitative measure of injury. Because of the relatively high level of resistance of St. Augustinegrass to  $\text{SO}_2$ -induced injury, measurement of leaf blade Chl content could not be used to quantify injury in this study.

The chemistry of  $\text{SO}_2$  in the environment (formation of the bisulfite ion) allows laboratory exposures of leaf discs to solutions containing bisulfite to be used as models of  $\text{SO}_2$ -plant interactions. The quantitative measurement of ethane production following an exposure to bisulfite has been well correlated with susceptibility to  $\text{SO}_2$ -induced injury in the Cucurbitaceae family. The ethane produced by excised leaf sections of St. Augustinegrass, when exposed to bisulfite in this study, was congruent with levels reported in the literature for  $\text{SO}_2$ -resistant plants.

The growth habit of Floratam may have been largely responsible for its relative (within the species) susceptibility to  $\text{SO}_2$ -induced visible injury and growth inhibition. Its greater canopy vertical growth rate, limited stolon growth, and greater dry weight per pot may have reduced carbohydrate levels (on a dry

or fresh weight basis) relative to the other cultivars, and thus reduced the energy available to repair enzymes needed by  $\text{SO}_2$ -stressed tissue. The greater vertical growth rate may have resulted in the shading of lower leaves and limited  $\text{CO}_2$  fixation near the bottom of the canopy. The limited stolon growth reduced the horizontal area available for the interception of light, and hence, photosynthesis would have been reduced on a per pot basis. The greater dry weight per pot and also greater vertical growth rate would have increased the consumption of photosynthate for structural purposes on a ground area basis which may have limited the energy available to enzymes responsible for repair of injured tissue. Perhaps, if Floratam were slower growing and more horizontal in growth habit, it would be better able to resist  $\text{SO}_2$ -induced injury.

The sulfur nutrition/metabolism of St. Augustinegrass will certainly affect its response to atmospheric  $\text{SO}_2$ . To date, no studies concerning the sulfur nutrition of St. Augustinegrass have been reported, which limits speculation as to its affect on  $\text{SO}_2$ -induced injury.

Although there was a significant interrelationship observed between the destruction of Chl and cell death (as quantified by ethane production) as a consequence of exposure of excised leaf sections to bisulfite and injury to whole plants by atmospheric  $\text{SO}_2$ , the most striking interrelationship was found between the visible injury sustained by plants exposed to acute  $\text{SO}_2$  levels,

and the growth inhibition (and also promotion) of plants exposed to long-term, low-level  $\text{SO}_2$ . Other cultivars and species should be tested with a similar model (such as that expressed by equation number 8).

The specific conclusions that can be drawn from these investigations include the following:

- 1) St. Augustinegrass should be classified as a  $\text{SO}_2$ -resistant species based on the results of the acute  $\text{SO}_2$  exposure portion of these investigations.
- 2) Cultivars vary in susceptibility to  $\text{SO}_2$ -induced injury among cultivars within the St. Augustinegrass species for both chronic and acute exposures.
- 3) Of the cultivars investigated, Floratam is the most susceptible to both acute and chronic  $\text{SO}_2$ -induced injury.
- 4) The growth of Floratam may be inhibited by  $\text{SO}_2$  concentrations currently realized in the field.
- 5) St. Augustinegrass cultivars, Raleigh, Seville, and Texas Common, were not detrimentally affected by the chronic  $\text{SO}_2$  exposures used in this study.
- 6) St. Augustinegrass cultivars Raleigh and Seville were extremely resistant to acute  $\text{SO}_2$ -induced visible injury, and may be beneficially used in resistance mechanism studies.
- 7) The "bisulfite/ethane system" was effective in predicting the  $\text{SO}_2$ -resistance of the St. Augustinegrass species, as well as cultivar resistance differentials.

- 8) Additional research investigating the effects of chronic  $\text{SO}_2$  levels on the growth of St. Augustinegrass is warranted.

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