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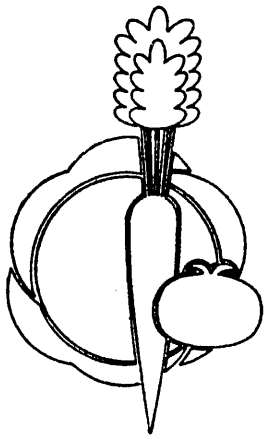

Final Report
August 26, 1985
For
ARB Agreement A9-050-31, A1-070,33,
A2-117-33, and A2-117-33 Amendment 1

DEPARTMENT OF LAND, AIR
AND WATER RESOURCES AND THE
DEPARTMENT OF VEGETABLE CROPS
UNIVERSITY OF CALIFORNIA, DAVIS, CA 95616

FINAL REPORT

to the

California Air Resources Board

Agreements No. A9-150-31
A1-070-33
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A2-117-33, Amendment 1

EFFECTS OF SO_2 AND OZONE ON CROP PHYSIOLOGY AND PRODUCTIVITY

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August 26, 1985

1. ABSTRACT

The aim of this project was to determine the primary plant mechanisms that are related to crop yield losses caused by SO_2 and ozone in California. Growth and productivity of several crop cultivars were related to physiological (stomatal conductance, plant water status, leaf membrane leakage, ethylene production) and biochemical (lipid peroxidation in chloroplasts) parameters. SO_2 behavior in soils was also studied.

In soils SO_2 showed little penetration and was rapidly oxidized to sulfate.

In laboratory studies seed germination was not sensitive to SO_2 and ozone but root growth was sensitive, particularly in tomato. SO_2 inhibited pollen tube growth; ozone had no effect on pollen viability. Differential SO_2 sensitivity in corn cultivars was not determined by stomatal SO_2 uptake but rather related to cellular uptake and detoxification. SO_2 induced ethylene production in a sensitive corn cultivar without visible injury. In bean cultivars sensitivity to ozone (but not to SO_2) was correlated with stomatal uptake, but both gases stimulated membrane leakage unrelated to differential cultivar sensitivity. Spinach chloroplasts exposed to SO_2 (sulfite) produced ethane and oxidized sulfite to sulfate. SO_2 damage in chloroplasts is mediated by lipid peroxidation.

In a field study an ozone-sensitive bean cultivar was grown in field chambers at low-level, long-term ozone exposure. Ozone decreased plant growth and pod yield, and lowered leaf water potential prior to bloom. A possible interaction between ozone and water stress was inconclusive. The variability in the physiological data were possibly related to air flow characteristics in the field chambers.

2. ACKNOWLEDGMENTS

Dr. Shang-Fa Yang, Department of Vegetable Crops, University of California, Davis, served as Co-Principal Investigator for Agreement Nos. A9-150-31 and A1-070-33; he was in charge of the biochemical studies on this project (see Chapter V, Part A). We acknowledge his contributions to this project. We also thank the following key personnel for their devoted assistance in this project: Ms. K. Barnes, Mr. L. Kent, Dr. D. Mock, Dr. G. Peiser, and Dr. D. Rush.

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3. DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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7. SUMMARY AND CONCLUSIONS

1. Behavior of SO_2 in Soil

The collective data from our studies on seed germination and root growth of bean plants in potting media, and the rapid decline of SO_2 in the soil atmosphere during incubation indicate that the soil environment was not harmed by SO_2 deposition. These data were obtained with soil reactions considered not acidic (pH 7.0 or higher) and included soil textures from sandy loam, to silt and clay loam with low concentrations of organic matter (less than 1%). It should be noted that our studies were very short-termed. Over a long time span light texture soil (sand) can more readily become acidic than heavier texture soil (clay) as a result of continuous exposure to SO_2. SO_2 is rapidly converted to SO_4^{2-} at the soil surface. Prolonged usage of acid forming fertilizer, such as ammonium sulphate, $(NH_4)_2SO_4$, has effected a lowering of the pH-reaction of many sandy soils in the San Joaquin Valley of California. The reaction of soils under cultivation for crop production can be reasonably and economically controlled by applications of limestone (crushed $CaCO_3$). However, vast areas of less accessible forest soils could not be easily limed nor would it be economically feasible. Intrusion of SO_2 (termed acid rain) could cause the soil reaction to become more acidic accompanied by a gradual loss of soil nutrients and a decline in overall soil fertility. However, some soils, deficient in sulphur, would show an improvement in general fertility.

2. Tissue Development and Plant Yield Response

a. Laboratory studies

Seed and/or pollen of several species of plants (alfalfa, corn, cucumber, pumpkin, squash, and tomato) reported to display varying sensitivity to either SO_2 or O_3 were exposed to levels of gases alone and in combination under controlled temperature conditions of 25°C to determine tissue growth responses.

Seed germination was unaffected by exposure to levels of SO_2 ranging up to 1.5 $\mu\ell\ \ell^{-1}$ and O_3 at 0.14 $\mu\ell\ \ell^{-1}$. However, marked

differences in seedling root growth occurred. Tomato root growth was the most sensitive to SO_2, with the greatest response occurring when exposed at the third and fourth day after germination. Cucurbits (cucumber, squash, and pumpkin) were less sensitive to SO_2. They displayed greater variability in response than tomato. Of the three corn cultivars studied root elongation of NK 51036 was less sensitive to SO_2 than were Bonanza and NC+59. Greater root growth retardation occurred with SO_2 alone than where O_3 was included.

Variability in seedling root growth was in part due to the germination media. Root growth apparently was adversely affected by the absorption of SO_2 in the unbuffered distilled water that resulted in an increase in H^+ (lower pH), whereas in the MES-buffered media the formation of HSO_3^- was likely. In general, as the dose rate increased root growth declined.

Pollen germination was apparently unaffected directly by SO_2. However, a change in media pH did effect lower germination of tomato pollen in unbuffered media. In both unbuffered and buffered media pollen tube growth of alfalfa, cucumber, and tomato decreased with addition of SO_2. The introduction of O_3 had little or no direct effect on pollen viability.

The impact of SO_2 on seedling root growth and pollen tube growth in unbuffered media is due to an increase in H^+ (lower pH) whereas in buffered media the formation of HSO_3^- is implicated. The magnitude of tissue response is dependent upon exposure time and level of SO_2, and plant species sensitivity. These findings may be useful in screening quickly plant species cultivar sensitivity to pollutants.

b. Field study

In the summer of 1984 snap bean cultivar Blue Lake Stringless 290, reported sensitive to O_3, was grown (at two soil moisture conditions) in field chambers and outdoor in the field to ascertain the influence of daily 5-hour chamber fumigation with a low level of O_3 between 0.07 and 0.10 $\mu\ell\ \ell^{-1}$ upon plant productivity. Plant development was measured at three stages of growth, pre-bloom, bloom, and at mature bean pod harvest.

A marked decline in plant growth with time and lower total fresh green pod yield was due primarily to daily exposure of plants to O_3.

Some decline in plant productivity was due to chamber environment and to soil moisture status. At harvest plants that had been treated with O_3 had developed 25% less leaf area and yielded about 41% lower fresh green pod weight compared with nontreated chamber and outdoor grown plants.

A gradual change in foliar color from a dark to yellowish green of plants exposed to O_3 was indicative of chlorophyll degradation that most likely led to a lowering in photosynthetic efficiency and carbohydrate production.

Fresh green pod yield was lower at a mean soil moisture tension of -0.10 MPa than at -0.05 MPa whether grown in chambers or in the field. The decline in fresh green pod yield of O_3 treated plants was about half at -0.05 MPa and about one-third at -0.10 MPa mean soil moisture tension compared with the pod yield of plants not exposed to O_3. The lowest fresh green pod yield was found with plants exposed to O_3 yet the pod yield was the same at both mean soil moisture tensions. This response suggests that plants at the more optimal soil moisture condition maintained a longer period of stomatal conductance for O_3 penetration. Although the gradual decline in foliar color was a symptom of O_3 damage, it was not clearly shown that leaf water potential and/or stomatal function was different among plants between the two soil moisture tensions which could account for the decline in plant productivity.

Despite the limited number of plants available for plant growth and pod yield measurement it has been shown that plant productivity is adversely affected by low level O_3 fumigation for 54 days. It is likely that had plant exposure to O_3 continued the yield of beans would also have been lower with present cultural inputs. There was no indication that plant nutrition nor lack of adequate soil moisture limited plant growth. Further studies could ascertain the specific interactions of plant responses between low level O_3 exposure and plant water potential on plant productivity.

3. Physiological and Biochemical Processes

a. Laboratory studies

Laboratory studies using environment-controlled fumigation chambers were conducted with corn and bean cultivars that have been previously described to be differentially sensitive to ozone. The cultivars used were NC and B (O_3-resistant) and NK (O_3-sensitive) for corn, and BTS and FH (O_3-resistant) and BBL and S (O_3-sensitive) for bean.

The primary aim of the studies on corn was to characterize the physiological responses to SO_2 of the three cultivars known to differ in ozone sensitivity, at two distinct developmental stages, i.e., young seedlings without functional stomata, and vegetative plants with green leaves and stomata, respectively. The results show that differential cultivar sensitivity to SO_2 is different from that to ozone. The O_3-sensitive NK was the most SO_2-resistant corn cultivar among the three studied. NK exhibited resistance to SO_2 at both the young seedling stage (determined as root growth) and the vegetative stage (determined as root and shoot fresh weight increase and visible leaf injury). The differential effect of SO_2 on stomatal conductance of the three cultivars was not correlated with their differential SO_2 sensitivity expressed as growth or injury. However, after long-term, low-level SO_2 fumigation, only the SO_2-sensitive cultivars NC and B showed an increase in leaf water potential. Thus, an increase in leaf water potential may serve as a physiological marker for SO_2 sensitivity in corn, but this possible application has yet to be ascertained under field conditions.

The studies on corn demonstrate that in this crop species cultivar differences in SO_2-sensitivity are not determined by differential uptake of SO_2 through the stomata. Differential SO_2 uptake at the cellular level and/or SO_2 detoxification may be more important factors in determining this plant's sensitivity to SO_2.

Ethylene production has been suggested as a convenient indicator of stress. It was tested whether ethylene production in the three corn cultivars is correlated with differential SO_2 sensitivity. SO_2 at a level of 0.3 $\mu\ell\ \ell^{-1}$ did not stimulate ethylene production, but at the level of 0.5 $\mu\ell\ \ell^{-1}$ SO_2 stimulated ethylene production from

cultivar NC which tended to be the most SO_2-sensitive corn cultivar in terms of fresh weight increase (0.5 $\mu\ell\ \ell^{-1}$ SO_2 did not cause visible injury). Thus, ethylene production appears to be an indicator of SO_2 stress at levels below those that cause visible injury; moreover, in corn ethylene production was only stimulated by SO_2 in the cultivar most sensitive to this air pollutant.

The experiments on the four bean cultivars known to differ in ozone sensitivity aimed at determining whether effects of ozone and SO_2 on stomatal conductance and ion leakage from leaf cells were correlated with differential sensitivity of the cultivars to the two air pollutants. They showed that ozone sensitivity in these bean cultivars was related to O_3-induced stomatal opening and therefore related to high rates of ozone uptake. Thus, an increase in stomatal conductance may potentially serve as a physiological marker for ozone sensitivity in bean, but this possible application could not be confirmed in the one year field study (see below under 3.b.). SO_2 at low levels did not cause visible injury in bean, and the observed SO_2 effects on stomatal conductance were not correlated with differential SO_2-sensitivity. In terms of ion leakage from leaf cells the cultivars showed differential responses to ozone and SO_2, applied singly, that were not related to differential air pollutant sensitivity. Stimulation of ion leakage by SO_2 has not been demonstrated before. Ozone and SO_2 in combination stimulated ion leakage from all four cultivars; addition of calcium ions protected the leaf membranes somewhat from the pollutant-induced membrane leakage. It would be interesting to test whether fertilization with lime or other calcium salts (e.g. gypsum) of soils that are not dominated by calcium could protect the foliage of crops from air pollutant-induced membrane leakage under field conditions.

It has been demonstrated that SO_2 can induce peroxidation of membrane lipids which can be experimentally determined by the formation of ethane. Chloroplast membranes, the site of photosynthesis, have been shown to be damaged by SO_2. Using spinach chloroplasts exposed to sulfite <u>in vitro</u>, our study showed that ethane formation caused by sulfite required light and occurred with concomitant oxidation of sulfite to sulfate, thus indicating that SO_2 damages

chloroplasts via lipid peroxidation, resulting in ethane formation. A biochemical mechanism for this sulfite-induced lipid peroxidation is proposed (see Chapter V, Part A, Figure V.3).

b. Field study

In the summer 1984 the ozone-sensitive bean cultivar BBL was grown in the field at two soil-moisture regimes and exposed to long-term, low-level ozone (0.07 to 0.1 $\mu\ell\ \ell^{-1}$ O_3 for 5 hours daily) in field chambers. At the developmental stages pre-bloom, bloom, and mature pod harvest the effects of ozone on the physiological parameters stomatal conductance, leaf water potential and ion leakage from leaf cells were assessed. The data show a considerable degree of variability. Long-term exposure to ozone had no significant effect on stomatal conductance, but stomatal conductance in well-watered plants exceeded that of water-stressed plants. Plants at the pre-bloom stage showed lower (more negative) leaf water potentials due to ozone treatment than control plants, and this was the case at both soil water regimes. However, there were no significant effects of ozone on leaf water potential at bloom and mature pod harvest. The decrease in leaf water potential caused by ozone in plants prior to bloom may be a useful physiological marker for ozone sensitivity in vegetative bean plants, provided this effect turns out to be consistent in additional field studies. Ozone had a significant effect on ion leakage from leaf cells only at bloom where ion leakage was stimulated, particularly in water-stressed plants. The possible interaction between ozone effects and water stress on ion leakage needs to be investigated further.

The field experiment yielded very variable results relative to the measured physiological parameters. The air flow characteristics of these field chambers may have effects on water loss characteristics of both plants and soil, and thus obscure the interpretation of ozone-soil water regime interactions. An additional field-chamber study is indicated before the effects of ozone on the measured physiological parameters in bean plants can be fully evaluated and their implications assessed.

8. RECOMMENDATIONS

We propose that another field chamber study relative to ozone be initiated with at least two growing seasons and more chambers to decrease variability of the results. In the proposed study the possible interaction with other environmental stresses (drought, salinity, heat) should be evaluated.

Ethylene production may serve as a physiological marker in screening crop cultivars for differential air pollutant sensitivity. Our study indicated such a possibility for differential SO_2 sensitivity in corn cultivars. An additional focused investigation needs to be made before one can make a general recommendation on the use of ethylene as a marker for air pollutant sensitivity.

In this study we found that calcium protected leaf membranes from air-pollutant induced membrane leakage. We recommend testing whether fertilization with lime or possibly gypsum of soils which are not dominated by calcium could protect crop foliage from air pollution injury.

9. BODY OF REPORT

Chapter IA. Field Chamber Characterization

IB. Characterization of Seedling Exposure Chambers (with 15 figures)

Chapter II. Penetration and Oxidation of Sulfur Dioxide in Soil (with 2 tables and 1 figure)

Chapter III. Comparative Development: Tomato and Cucurbit (with 3 tables and 6 figures)

Chapter IV. Comparative Development: Corn (with 8 tables and 5 figures)

Chapter VA. Ethane Formation from Chloroplasts Exposed to SO_2 - A Measure of Lipid Peroxidation

Chapter VB. Ethylene Formation from Corn Exposed to SO_2 (with 5 tables and 3 figures)

Chapter VI. Snap Bean (Phaseolus vulgaris L.) Plant Growth and Yield Response to Low Level Ozone Exposure (with 6 tables and 1 figure)

Chapter VII. Growth Chamber and Field Chamber - Physiological Processes (with 1 table and 27 figures)

CHAPTER IA. Field Chamber Characterization

1. Introduction

A number of environmental factors are known to affect plant response to air pollutants (Taylor, 1974; Tingey and Taylor, 1982); it is therefore imperative to minimize environmental differences when exposing plants to air pollution treatments. In field investigations open-top chambers with single-pass air flow systems were shown to have temperatures and relative humidities similar to those found in adjacent ambient conditions (Heagle et al., 1973; Buckenham et al., 1981). Difficulties with open-top field chambers may arise from the following factors: intrusion of ambient air (Kats et al., 1976; Buckenham et al., 1981), reduction of solar radiation (Olszyk et al., 1980), variable rainfall patterns (Heagle et al., 1973; Mandl et al., 1973), air movement characteristics (Unsworth et al., 1984), and evaporative water loss (Olszyk et al., 1980). Temperature differences between chambers and ambient air above 35°C reached 2°C (Mandl et al., 1973; Heagle et al., 1979; Howell et al., 1979). Variability within chambers by any of the above mentioned parameters may further complicate interpretation of experimental results.

Minimization of chamber effects and of variability within chambers is essential for the accurate assessment of plant responses to air pollution stress in such chambers. The degree to which the aforementioned factors may influence results will depend upon the microclimate and chamber design. Open-top field chambers have usually been designed after those of Heagle et al. (1973). A truncated-cone (frustum) was added to reduce ambient air intrusion (Buckenham et al., 1981). Summer rainfall may be discounted as a factor in the Mediterranean-type climate of Davis, CA. The variability reported for evaporative water loss (Olszyk et al., 1980) between the north and south chamber quadrants was of concern to us because our investigation attempted to evaluate the effect of plant water status on response to air pollution stress. It should be noted that soil moisture in the cited investigation depended exclusively on rainfall. We attempted to overcome this complication by using deep drip irrigation and by

placing our wet and dry soil treatments in the east and west halves of the field chambers.

Reduced and variable solar radiation within field chambers is a consequence of the material chosen to enclose the chamber and the shading by the chamber frame. Field chambers are generally enclosed in fiberglas (Mandl et al., 1973) or polyvinyl chloride (Heagle et al., 1973). These materials were found to transmit 95% (Mandl et al., 1973) and 98% (Olszyk et al., 1980) of direct overhead radiation, respectively. Shading by the aluminum supports should be minimal because of the movement of the sun and the small size of the supports.

Heat buildup in the chambers when ambient temperatures exceeded 35°C was a major concern in this investigation. The addition of a second blower to each chamber to increase the rate of air exchange was therefore proposed and tested.

This investigation characterized two types of open-top field chambers, i.e., one- and two-blower designs, and compared the chamber environment with that of adjacent, ambient plots.

2. Materials and Methods

Four NCLAN-type open-top chambers were placed in the field (Fig. I.1a). The chambers had aluminum channel frames and were covered with clear 8 mil polyvinyl chloride. The dimensions of the chambers were 3.10 m in diameter and 2.95 m high. The uppermost panel was a truncated cone to prevent intrusion of ambient air on windy days.

Initial chamber characterization was carried out in spring 1984 on two chambers equipped with one or two "squirrel-cage" blower modules (Heat Controller, Inc., Model B20-36). Blowers were equipped with a fiberglass particulate filter and an activated charcoal filter (1000 cfm; Model W45, Westates Carbon, Los Angeles). The blowers were attached to the lower panel of the chambers by a PVC umbilical cord with a diameter of 0.51 m. Air from the blowers inflated the perforated lower panel of the chamber and was distributed uniformly throughout the chamber. Air exchange rates were 1.5 and 3.1 min^{-1} for the one- and two-blower chambers, respectively.

Copper-constantan thermocouples were initially placed at the center of the two chambers and at an adjacent outside site at 0.1 and 1.0 m above the surface of the bare soil. Inverted styrofoam cups were placed above the thermocouples for shading. Temperatures were recorded hourly on a data acquisition system (Model 9302; Moniter Labs, San Diego, CA), for eight days. Temperatures were then recorded on a transect, at the same heights, from the northwest to the southeast in the chambers. Locations were at five points at the following distances (m) from the northwest edge of the chamber 0.46 (NW), 0.91 (NW CTR), 1.52 (CTR), 2.13 (SE CTR), and 2.59 (SE). Soil temperatures were also recorded at a depth of 8 cm.

Following characterization of the two chambers described above, bean seeds were planted in all four chambers on June 13, 1984 and the lower panels of the chambers were removed until plant establishment (June 27). Two adjacent plots were also planted and treated in the same manner as the chamber plots. Ozone fumigation was initiated on June 29. Two chambers were fumigated with 0.07 to 0.10 $\mu\ell\ \ell^{-1}$ O_3 for five hours daily. Ozone was generated by passing oxygen (minimum purity 99.5%) through a silent arc ozonator (Model 03V10-0; Ozone Research and Equipment Corp., Phoenix, AZ). Ozone delivery was through nylon tubing (1/8" i.d.) to both blowers attached to each of the two chambers. Fumigations were between approximately 1100 and 1600 hours daily. The other two chambers were supplied with filtered air that contained approximately 0.02 $\mu\ell\ \ell^{-1}$ O_3 or less. The two outside plots were exposed to ambient ozone levels (see Fig. I.1b).

Ozone samples were taken at two heights (10 and 25 cm) in the center of the four chambers and in one of the adjacent plots. Air samples were pulled through Teflon tubing (1/4" o.d.) by a Thomas pump equipped with a Teflon diaphragm and routed through a twelve channel Scannivalve gas sampling system. Each channel was monitored for ten minutes and recorded on the data acquisition system. Ozone analysis was carried out by Dasibi Ozone Monitors (Models 1003PC and 1003AH) equipped with Teflon filters. On August 6 ozone sampling sites were moved to further characterize the ozone distribution in the fumigated chambers. One sampling tube was left in the center of the two nonfumigated chambers 25 cm above the soil. Sampling tubes in the

fumigated chambers were placed 0.6 m from the north and southeast walls.

Temperatures in the field chambers were recorded hourly at the center and 0.6 m from the north wall at 25 cm height throughout the growing season. Bean plants were harvested on August 21.

Another bean crop was planted September 5, 1984 in two chambers. The lower panels were left in place, unlike the previous planting. One chamber was fumigated, beginning September 27, with O_3 at the same dose as was applied during the summer. The other chamber was supplied with filtered air. Thermocouples were placed at five locations in the chambers specified previously for the northwest-southeast transect at a height of 50 cm. Ozone sampling tubes were placed at the same locations as the thermocouples in the fumigated chamber. One ozone sampling tube was placed in the filtered chamber and one at an adjacent location. Ozone samples were recorded every three minutes from each location. Fumigation was discontinued October 13.

Light measurements were made at canopy level in a chamber and an outside plot, hourly from 0900 to 1500 hrs. A light meter (Model LI-170; LI-COR, INC., Lincoln, NE) was used to measure photosynthetically active radiation (PAR; λ = 400-700 nm). Measurements were taken along an east-west transect in the chamber and the outside plot at the height of the plant canopy.

3. Results

Initial temperature characterization was carried out over bare soil in chambers equipped with one or two "squirrel-cage" blowers. Typical diurnal temperature patterns are shown in Figs. I.2 and I.3 at 0.1 and 1.0 m, respectively. Figure I.2 illustrates the beneficial effect of the two-blower chamber. During the period of highest temperatures, the two-blower chamber was 3°C below the one-blower chamber. It is interesting to note the difference in behavior between the ambient and chamber temperature patterns. Outside nocturnal temperatures at 0.1 m remained quite high relative to those of the two chambers. This could have dramatic effects on seedling growth and canopy establishment. This phenomenon was neither apparent at 25 cm (Fig. I.4) with a plant canopy present, nor at 50 cm (Fig. I.6) and

1 m (Fig. I.3) over bare soil. At 1 m above bare soil (Fig. I.3) ambient temperatures followed the temperature patterns in the chambers though at a lower level. Temperatures in the two-blower chamber were approximately 3°C lower than in the one-blower chamber during the hottest part of the day.

The above results led to our adoption of the two-blower chamber for our subsequent field investigation of ozone effects on bean plants. Our decision to remove the lower chamber panel until plant establishment was based on nocturnal temperature differences between ambient plots and chambers that may have affected plant growth, the nocturnal relative humidity, and the large difference in maximum temperatures observed in the afternoon.

Following crop establishment the temperatures in the chambers were slightly lower than those outside (Fig. I.4). The temperature range among the four chambers is probably due to the differential growth of plants in the chambers. The chambers supplied with filtered air (chambers 1 and 3, Fig. I.4) had the lowest maximum temperatures. This was most likely due to shading or evaporative cooling by the more developed plant canopies in these two chambers. Nocturnal temperatures in the chambers and outside were similar at 25 cm height in a plant canopy (Fig. I.4).

Soil temperatures at 8 cm depth during a day are compared in Fig. I.5. Over a 24 hr period the temperature range for ambient and chamber conditions was only 4 and 6°C, respectively. The mean daily temperature under both conditions was nearly identical even though the pattern of variation throughout the day was somewhat dissimilar (Fig. I.5).

Temperature variation within a chamber on a diurnal basis was examined during the early autumn over bare soil (Fig. I.6) and a plant canopy (Fig. I.7). Temperatures were measured at a height of 50 cm. Over bare soil, chamber temperatures were considerably higher than those under ambient conditions. The temperature range in the chamber, when measured over bare soil, was less than 1°C from 0000 to 1000 hrs and from 1800 to 2300 hrs (Fig. I.6). The maximum temperature differential within the chamber was 2.7°C when the average temperature in the chamber was 40.9°C. Therefore the maximum temperature

variation within the chamber was only 7%. Temperature variation over a plant canopy followed a similar diurnal pattern relative to that over bare soil. The maximum variation over the plant canopy was 11% at a mean chamber temperature of 30.7 (Fig. I.7).

Mean temperature variation over a period of seven days is illustrated in Fig. I.8. Temperatures within the chambers varies by less than 10% with a mean temperature of 22.5°C for this period. Mean ambient temperature for the same period was 21.7°C.

Ozone concentrations throughout a typical fumigation day are illustrated in Fig. I.9. Chambers receiving filtered air had ozone concentrations up to 0.02 $\mu\ell\ \ell^{-1}\ O_3$ throughout the day (Fig. I.9). Ambient ozone concentrations reached a maximum of approximately 0.05 $\mu\ell\ \ell^{-1}\ O_3$ between 1500 and 1600 hrs on this particular day. Ambient ozone concentrations followed this general pattern throughout the experimental period, but the maximum concentration reached depended on meteorological conditions. Ozone concentrations in the fumigated chambers varied from 0.080 to 0.089 $\mu\ell\ \ell^{-1}\ O_3$ during the exposure period with a mean of 0.0825 $\mu\ell\ \ell^{-1}\ O_3$. The distribution of ozone in the two-blower field chamber during fumigation may be seen in Fig. I.10. The exposure period mean of ozone concentration for the three points in the chamber was 0.083 $\mu\ell\ \ell^{-1}\ O_3$. Variation among the points for the full fumigation period was 0.005 $\mu\ell\ \ell^{-1}\ O_3$. For a given sampling sequence, variation within the chamber ranged from 4 to 18% of the mean ozone concentration.

Mean PAR was 20% less in the chambers than outside between 0900 and 1500 hrs. The overall level of PAR in the chamber was significantly lower than in the outside plot (Fig. I.11). Time of the day had also a significant effect on the amount of incident PAR at the field site, as expected. There were no significant differences in PAR for location on the transects or any interactive terms (chamber x time, chamber x transect location, and time x location).

4. Discussion

The initial focus of our study was to characterize two types of open-top chambers and how these characteristics might affect plant growth. One chamber was constructed with a single "squirrel-cage"

blower and another with two blowers on opposite sides of the chamber. We compared temperatures, over bare soil, between the two chamber types and with ambient conditions. The two-blower chamber was found to be approximately 3°C cooler than the one-blower chamber during the afternoon when temperatures were at a maximum (Figs. I.2 and I.3). Both chambers had daytime temperatures that were considerably above those in ambient conditions. This was not unexpected because of the absence of vegetation and dry soil conditions. Nocturnal temperatures in both chambers were nearly identical. Surprisingly, outside nocturnal temperatures at 10 cm remained much higher than those in the chambers. This was probably due to the radiation of heat by the soil and the lack of atmospheric mixing at this height above the soil. Turbulence created by the blowers in both chambers appeared to be sufficient for mixing the layer of air adjacent to the soil. Comparing the nocturnal temperatures at 0.1 m (Fig. I.2) and 1.0 M (Fig. I.3) supports this suggestion. Temperatures were nearly identical at both heights in the chambers, while the outside temperatures at 0.1 m were much higher than those at 1.0 m (Figs. I.2,3).

We decided to use two-blower, open-top field chambers for two reasons. First, the ability of the two-blower chamber to maintain a more moderate temperature during the hottest part of the day was considered very important because of the high summer temperatures that are common in the Central Valley of California. Second, the greater air exchange capacity of a two-blower system was considered crucial in controlling the concentration of ozone during fumigations and preventing the intrusion of ambient air into our control chambers.

At the time of sowing it was thought prudent to remove the lower panel of the chambers and to turn off the blowers. This was considered essential for the survival of the high-temperature sensitive bean seedlings. Another factor was the phenomenon of higher nocturnal temperatures under ambient conditions than in the chambers. The temperature differential itself may have had variable effects on the young seedlings, and furthermore the water holding capacity of air at such divergent temperatures may be different enough to affect seedling establishment. For example, at 0100 hrs the ambient

temperature was 27°C at 0.1 m while the chambers were at 15°C. The difference in relative humidity, at the same wet-bulb depression, for these two temperatures is 18%. This could have dramatic effects on stomatal behavior in the early morning hours before temperatures become more equal. Of course, this could possibly affect the water relations of the seedlings and subsequently of growth.

After the plant canopy was established and fumigations initiated, temperatures were examined for uniformity among the chambers and divergence from ambient conditions (Fig. I.4). With an established plant canopy chamber temperatures were somewhat below those outside during the afternoon period of maximum temperatures. Nocturnal temperatures were similar in all four chambers and outside. Daytime temperatures in the chambers were quite variable with differences ranging from 3 to 5°C (Fig. I.4). This variability was most likely due to shading within the chambers and differential plant development. Differential plant development would result in variable evaporative cooling from the canopy and the soil in a chamber. It should be noted that the two chambers receiving filtered air had the lowest maximum temperatures (Fig. I.4).

Soil temperatures throughout the day were fairly uniform (Fig. I.5). The mean temperatures for the day illustrated in Fig. I.5 were identical for the outside plot (26.2°C) and the chamber (26.1°C).

Following the harvest of the summer bean crop, two chambers were replanted on September 5, 1984 and further characterization of temperature and ozone conditions within the chambers was carried out. Unlike the previous experiment, seeds were sown in fully operational chambers. Seedling establishment did not appear to be adversely affected by this procedure but may have been related to the slightly lower temperatures in September. Furthermore, an outside plot was not used for comparison. The procedure described above is not recommended for stand establishment in field chamber studies, particularly where temperatures may exceed 40°C, as is often the case during the summer in the Central Valley of California.

Temperatures were monitored hourly at a height of 50 cm until flowering was well established and the experiment was terminated (October 13). Variability of temperatures within a chamber is

presented for two distinct conditions: over bare soil prior to seedling emergence (Fig. I.6) and over a well developed canopy (Fig. I.7). Temperature variation within a chamber was practically nonexistent at night and greatest between 1000 and 1700 hrs under both conditions. The uniformity of nocturnal temperatures within a chamber implies that the air dispersion system is creating a well mixed atmosphere in the chambers. The design of the dispersal system would most likely be the foremost factor in contributing to variable temperatures at night. Daytime chamber temperatures are subject to a number of factors that may affect uniformity of temperatures. In addition to the air dispersion system, factors affecting chamber temperature uniformity are: vegetation, soil moisture, and shading by the chamber. Vegetation may be considered both a biological and physical factor. The physical aspects of vegetation's effect on temperatures are interference with air dispersal and shading. Sampling temperatures above the plant canopy was an attempt to minimize these aspects. The biological aspect of vegetation, that is, evaporative cooling through transpiration, is minimal at night and was eliminated (Fig. I.6). The maximum temperature range within a chamber prior to seedling emergence was 6.6% of the mean chamber temperature (40.9) at that time. When vegetation was present (Fig. I.7) maximum temperature range was 11.0% of the mean (30.8). It is tempting to attribute the increase in chamber temperature variability to plant canopy characteristics, but complications arise from the difference in temperature on the two dates and, furthermore, the angle of incidence of the sun is lower in early October (Fig. I.7) than in early September (Fig. I.6). Plant canopy effects should not be discounted, however, but transpirational water loss and shading of the soil are undoubtedly important factors in the degree of uniformity.

For a seven day period, mean daily temperatures in two chambers averaged 0.8°C higher than those in ambient conditions. These results are in agreement with previous studies that utilized similar chambers (Heagle et al., 1979; Buckenham et al., 1981). Mean temperatures for seven days at various positions in two chambers are illustrated in Fig. I.8. The seven day mean temperature as recorded at seven locations for chambers 2 and 3 was 22.5 with variances of 0.4 and 0.6,

respectively. Mean temperature range within chambers was 1.1°C for chamber 2 and 1.8°C for chamber 3. Temperature variations were 4.9% and 8.0% of the mean chamber temperature for chambers 2 and 3, respectively.

Ozone concentrations for a typical day during the summer are shown in Fig. I.9. Intrusion of ambient air does not appear to be a serious problem in our control chambers. Details of ozone distribution in a chamber during a fumigation period (not the same as Fig. I.9) appears in Fig. I.10. The mean ozone concentration from seven sampling locations in the chamber was 0.082 ± 0.003 $\mu\ell\ \ell^{-1}\ O_3$. Only three locations are presented in Fig. I.10 to avoid confusion. Mean ozone concentrations for the fumigation period ranged from 0.078 to 0.085 $\mu\ell\ \ell^{-1}\ O_3$ among the seven sampling sites. Heagle and Philbeck (1979) recommended a mean deviation of less than 10% for low level pollution exposures. The ozone concentration variability presented above and in Fig. I.10 falls below this recommended level.

Incident PAR was 20% lower in the chambers than outside between 0900 and 1500 hrs. Olszyk et al. (1980) recorded a similar reduction but concluded that it was not large enough to reduce photosynthesis. No significant variation on an east-west axis was apparent in the field chambers. Olszyk et al. (1980) observed a higher level of radiation in the north quadrant than in the south quadrant of their field chambers.

The conclusions reached by this investigation suggest that the two-blower field chamber design is superior to the one-blower chamber in maintaining temperatures close to those of ambient conditions. Temperatures in the chambers and outside plots were similar in the presence of a developed plant canopy. Temperature variability among and within chambers is most likely because of the shading and reflectance properties of the chambers themselves and vegetation effects. Solar radiation (as PAR) was reduced within the chambers. Differences between the ambient and field chamber environments in incident radiation and air movement may result in growth effects. This may make economic loss assessment a difficult proposition and may confound physiological studies if caution is not exercised.

CHAPTER IB. Characterization of Seedling Exposure Chambers

1. Introduction

Air pollution studies of plants have focused on well established or mature plants which require full-sized fumigation chambers. We were interested in exposing germinating seeds and seedlings as well as flowers and pollen to low levels of SO_2 and O_3; we began by developing effective yet small chambers for short-term (up to one week) exposures. Since the chambers were small we found we could design experiments with three and four treatments yet still efficiently replicate chambers.

2. Materials and Methods

The chambers were glass chromatography jars fitted with a metal plate having one hole bored through the center. This hole was plugged by a rubber stopper with three 1/4" holes: influx, efflux, and sampling ports for each chamber's atmosphere. Two sizes of jars were tested, 9- and 16-liter, placed upright and placed on the side. The final set up consisted of twelve 16-liter chambers placed lengthwise.

The gas distribution system consisted of three gas cylinders containing SO_2:N_2 mixtures of 50, 100, and 200 $\mu\ell\ \ell^{-1}$ SO_2 and fitted with stainless steel pressure regulators in addition to in-line low pressure regulators. Filtered, compressed air was used for diluting the SO_2 to low concentrations, for control chambers with no SO_2, and for continuous flushing between daily four hour fumigations. Calibrated glass capillary tubes controlled the flow of SO_2 and air. Scrubbing towers for each SO_2:N_2 tank and the compressed air were used between the low pressure regulators and the capillary tubes to minimize changes in flow due to fluctuations in pressure (Fig. I.12). Since there was no fluctuation in influx concentration, a mixing vessel was not necessary.

Ozone treatments were controlled in the same manner as the SO_2. A gas cylinder of oxygen was connected to a Saunders ozonizer; ozone concentration was controlled by adjusting the ozonizer and the glass capillary tube. Ozone and SO_2 were combined after each gas was

diluted by the compressed air and prior to chamber delivery. There was no measure of possible NO_x formation.

All connective tubing was 0.64 cm (1/4 inch) TFE teflon. Delivery of gas within each chamber was through perforated tubing pinched closed at the end and positioned along the bottom of the chamber. Evenly spaced perforations (every 3 cm) provided the most even gas distribution within the chamber. Flow rate remained stable at 300 ℓ/hr or one exchange every 3.2 minutes in the 16-liter chambers. For the purpose of characterizing the chambers, SO_2 influx concentration was 0.75 $\mu\ell\ \ell^{-1}$.

Adsorption of SO_2 by the chamber walls was minimized by rinsing with 0.1 N HCl. Stands were placed in the chambers to support the experimental material 5 cm above the perforated delivery tube, restricting fumigant and air flow as little as possible. SO_2 and O_3 concentrations were easily monitored within each chamber by unplugging the sampling port and inserting a teflon sampling tube. A Dasibi measured ozone concentrations and a pulsed fluorescent SO_2 analyzer (TECO) measured SO_2 concentrations.

3. Results

Initial work with the chambers was done on the 9- and 16-liter chambers standing upright with SO_2 added from a point source positioned 3 cm above a flat filled with moistened vermiculite. Figure I.13 shows the height gradient in those chambers and also shows the adsorption of SO_2 in the presence of a moist medium. The tube influx concentration was 0.75 $\mu\ell\ \ell^{-1}$, but the ambient concentration ranged from 0.10 to 0.45 $\mu\ell\ \ell^{-1}$. With constant flow rate, chamber volume, and influx concentration, ambient concentration became dependent on duration of fumigation and on the volume and water holding capacity of the germinating media in the chamber (Fig. I.14). Ambient concentration increased with time for all media and was inversely correlated to media water holding capacity. Adsorption of SO_2 and the fate of SO_2 in soil is further discussed in Chapter II.

Further work with the chambers showed that turning the chambers lengthwise afforded a bigger working area; using the perforated delivery tube along the bottom of the chamber minimized the horizontal

concentration gradient (Fig. I.15). Figure I.15 also shows a consistent ambient SO_2 concentration among three replicated chambers.

4. Discussion

By using standard equipment for delivery of constant volumes of gas and small chambers, an inexpensive yet experimentally efficient fumigation system was constructed. Replication between chambers was acceptable, gradients were moderate, and ambient concentration was controlled through an understanding of adsorption rates of SO_2 by moist media. Furthermore, large experiments (three or four treatments replicated four or three times, respectively) could be conducted in a small space and with a minimum amount of equipment. Results of experiments conducted in these chambers are discussed in Chapters III and IV.

5. Summary

Fumigation chambers for small scale laboratory and large field experiments are described and characterized for variability within and among chambers.

Two designs of open-top field chambers were considered for use in the summer field experiments described in Chapters VI and VII. It was found that two-blower chambers were more effective in maintaining temperatures close to ambient than were one-blower chambers. Chamber temperatures were similar to ambient in the presence of vegetation. Temperature variation within and among the chambers was thought to be a function of plant canopy development. Photosynthetically active radiation was reduced by 20% within the chambers compared to adjacent outside areas. Slight ozone variability was found within the fumigated chambers, but this was thought to be due to the sequential sampling system and was well within the recommended guidelines suggested by Heagle and Philbeck (1979).

Small scale fumigation chambers were found to be experimentally efficient in terms of replication. Gradients were moderate within the chambers and fumigation concentrations could be adequately controlled through an understanding of adsorption rates of SO_2 by the moist media within the chamber.

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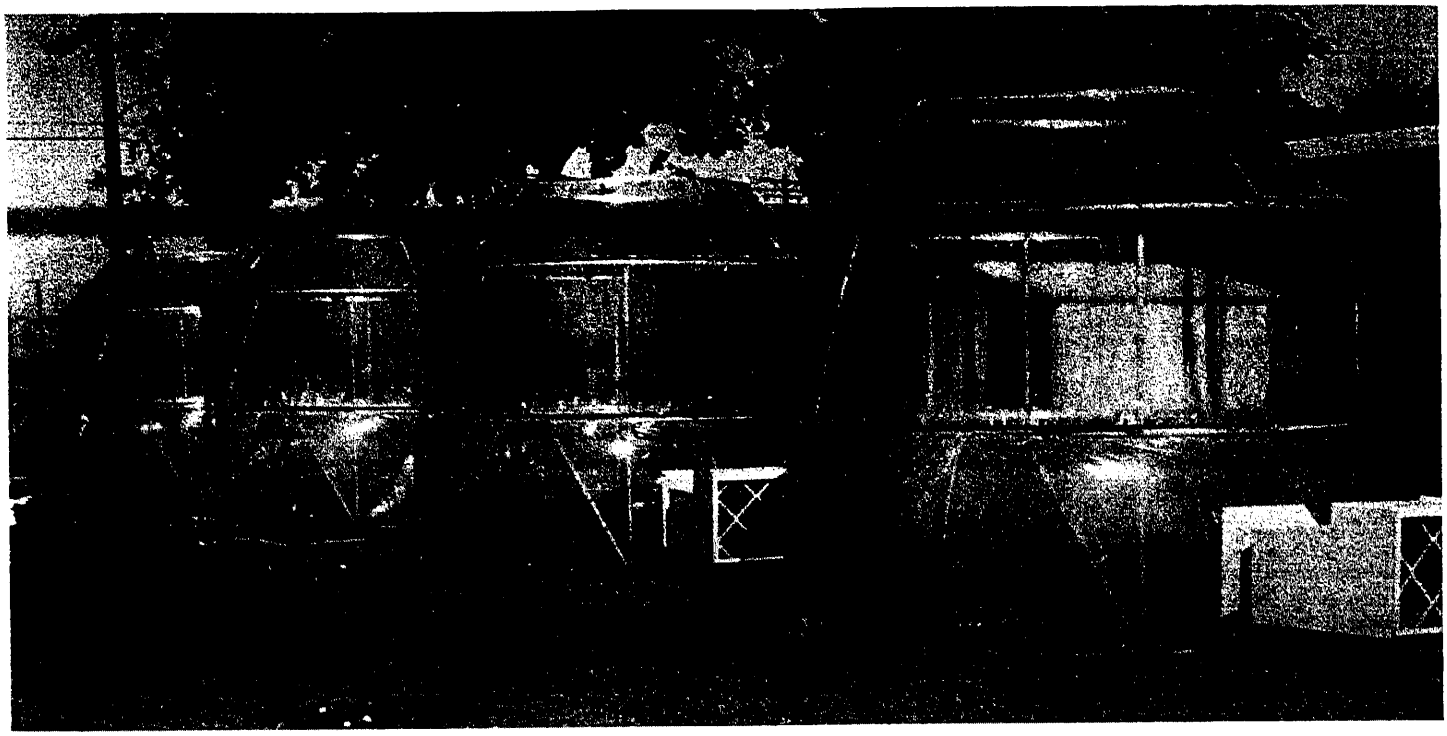

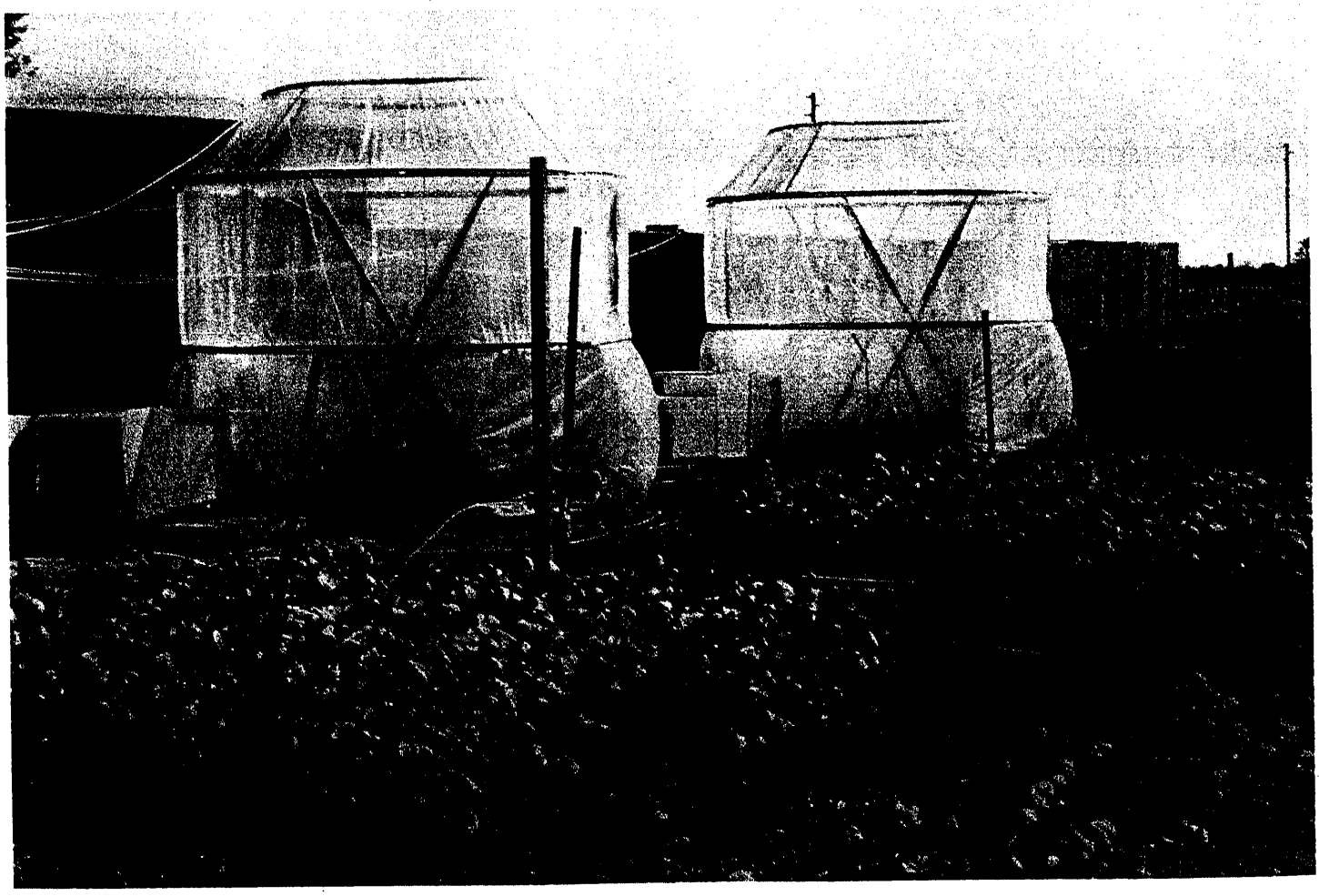

Figure I.1a Location of four open-top field chambers at the University of California, Davis.

Figure I.1b Open-top field chambers with ambient plots in foreground.

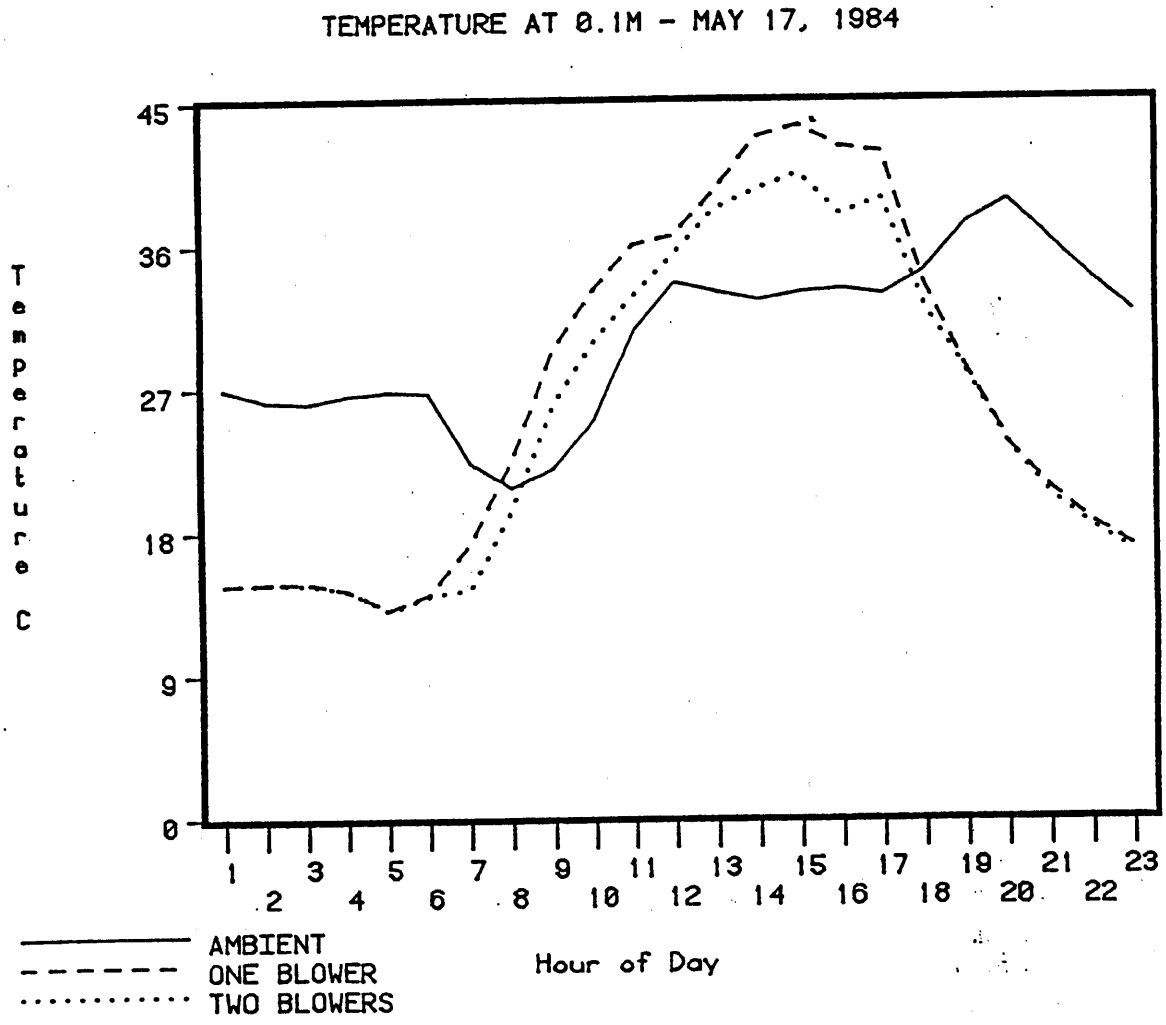

Figure I.2 Comparison of diurnal temperature pattern at 0.1 m above soil surface in ambient plot, one- and two-blower chambers.

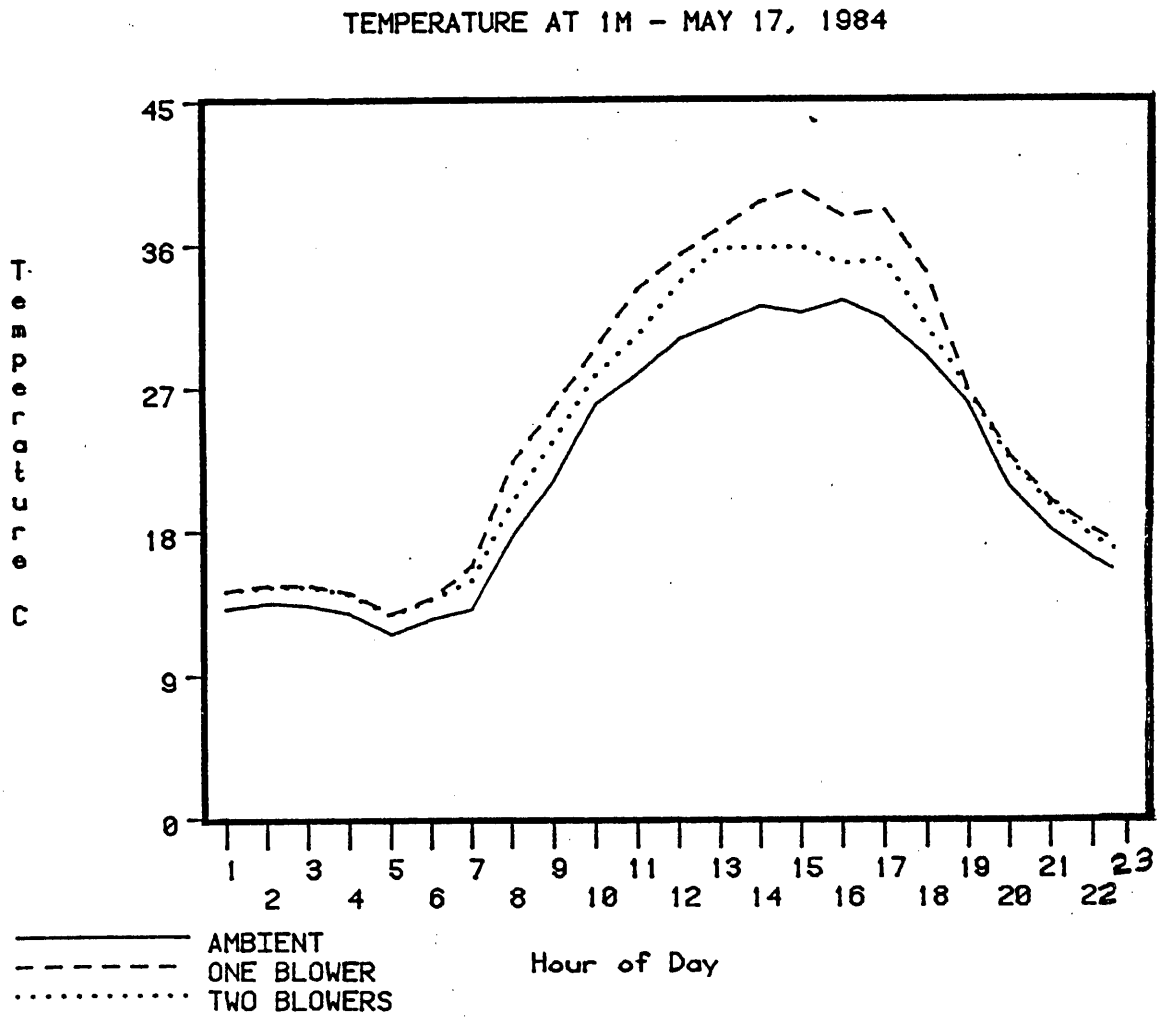

Figure I.3 Comparison of diurnal temperature pattern at 1 m above soil surface in ambient plot, one- and two-blower chamber.

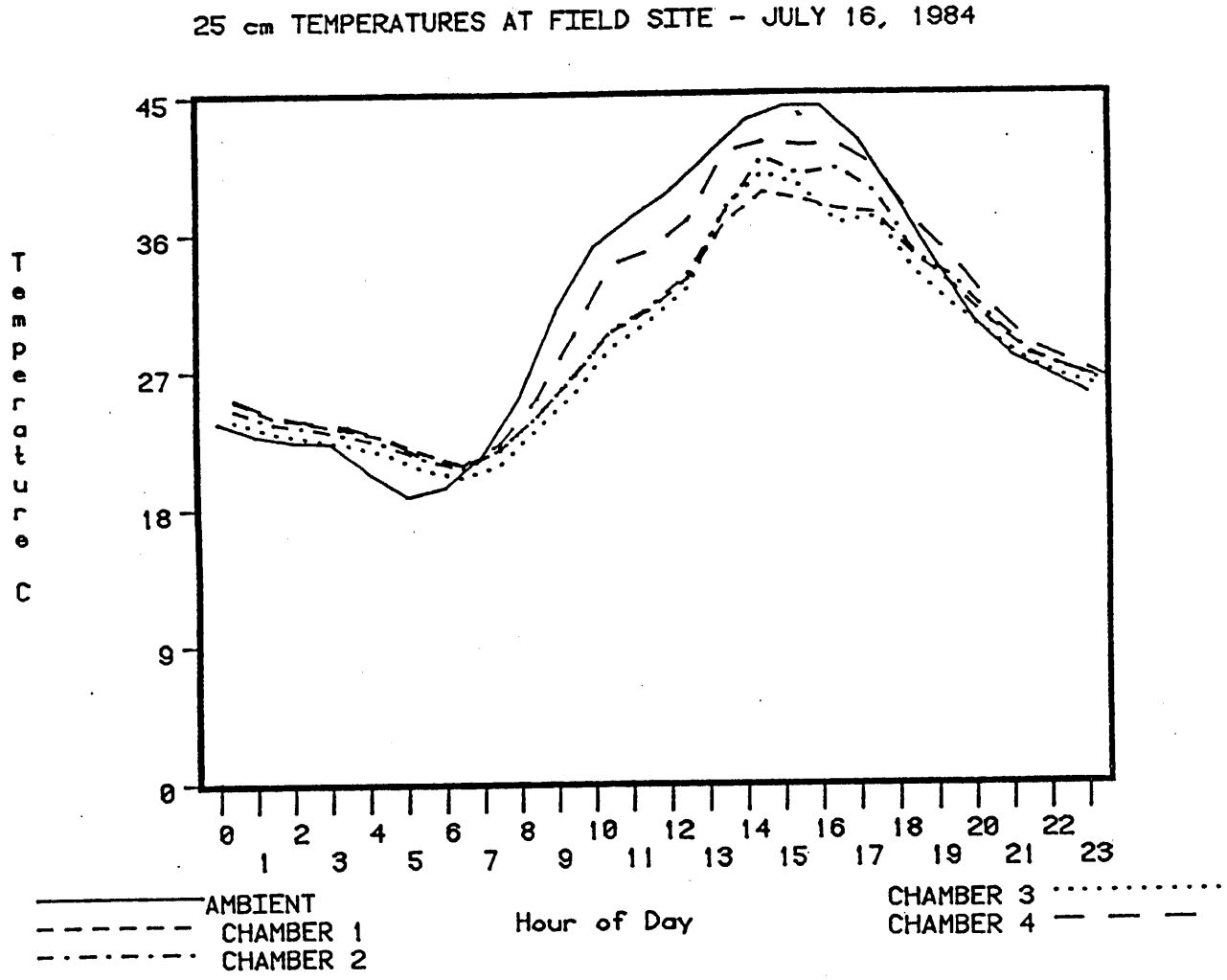

Figure I.4 Temperature comparison at 25 cm height among two-blower chambers in the presence of a developed plant canopy.

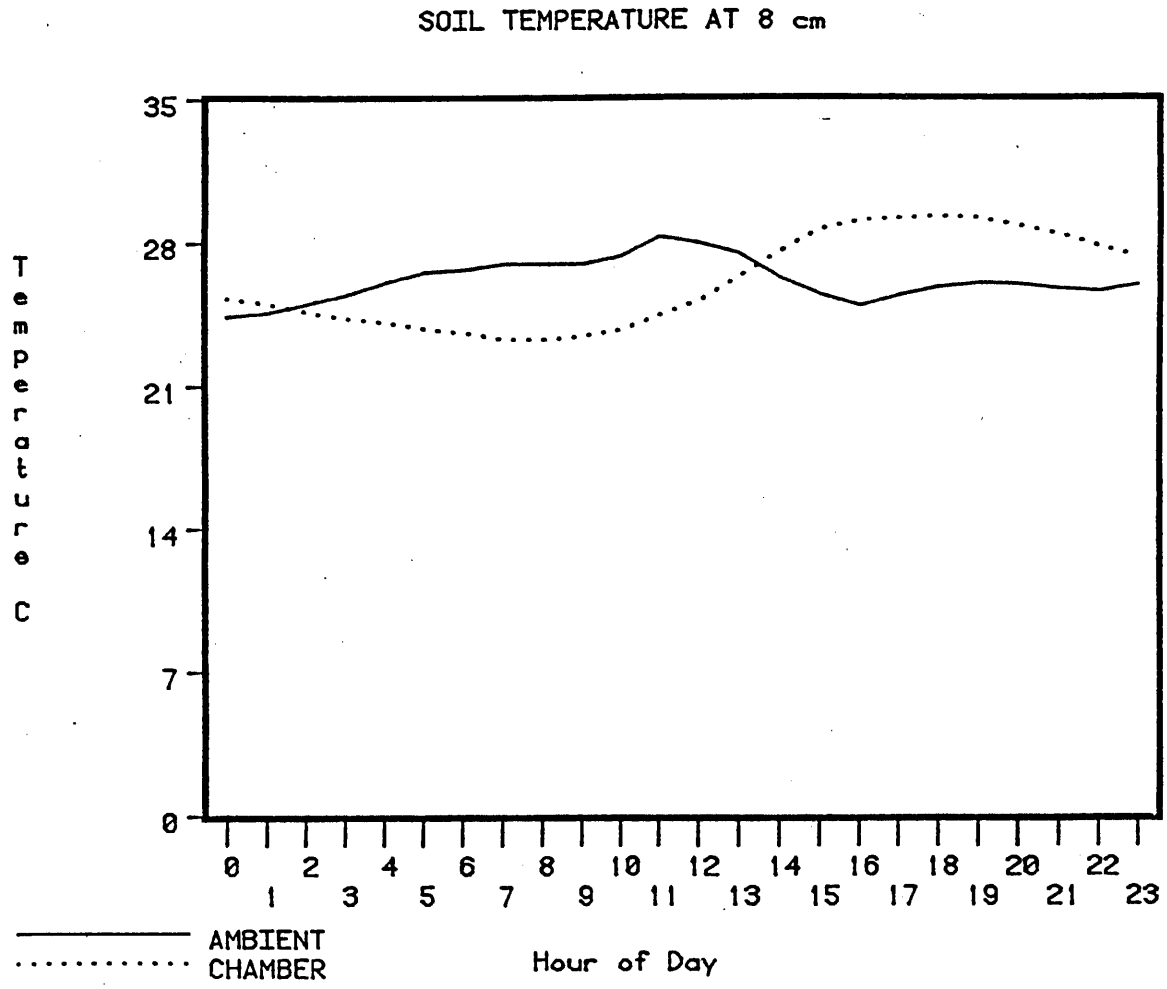

Figure I.5 Soil temperature patterns at a depth of 8 cm. Comparison of a two-blower chamber and ambient conditions.

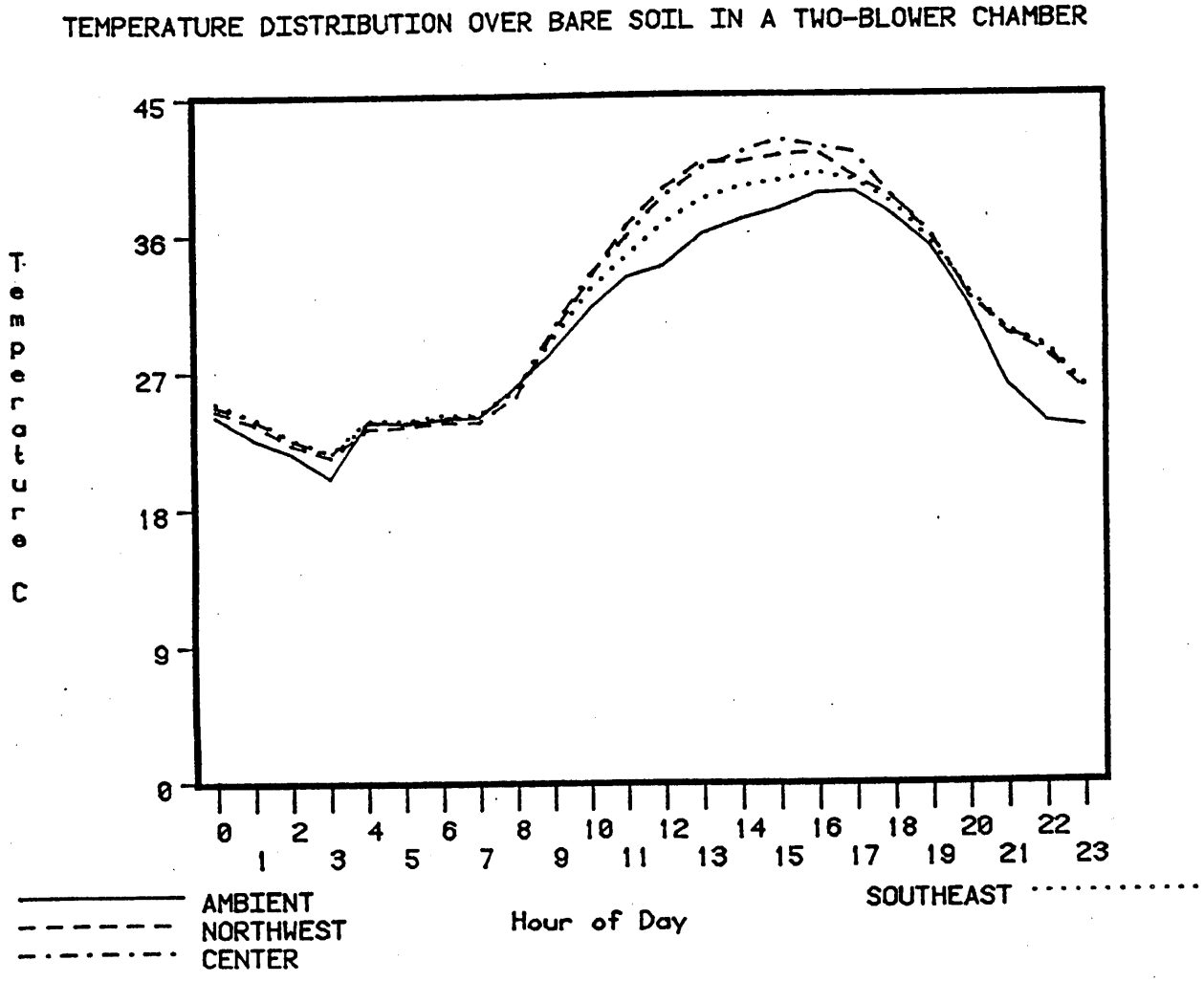

Figure I.6 Temperature variability within a two-blower chamber at 50 cm above surface of bare soil.

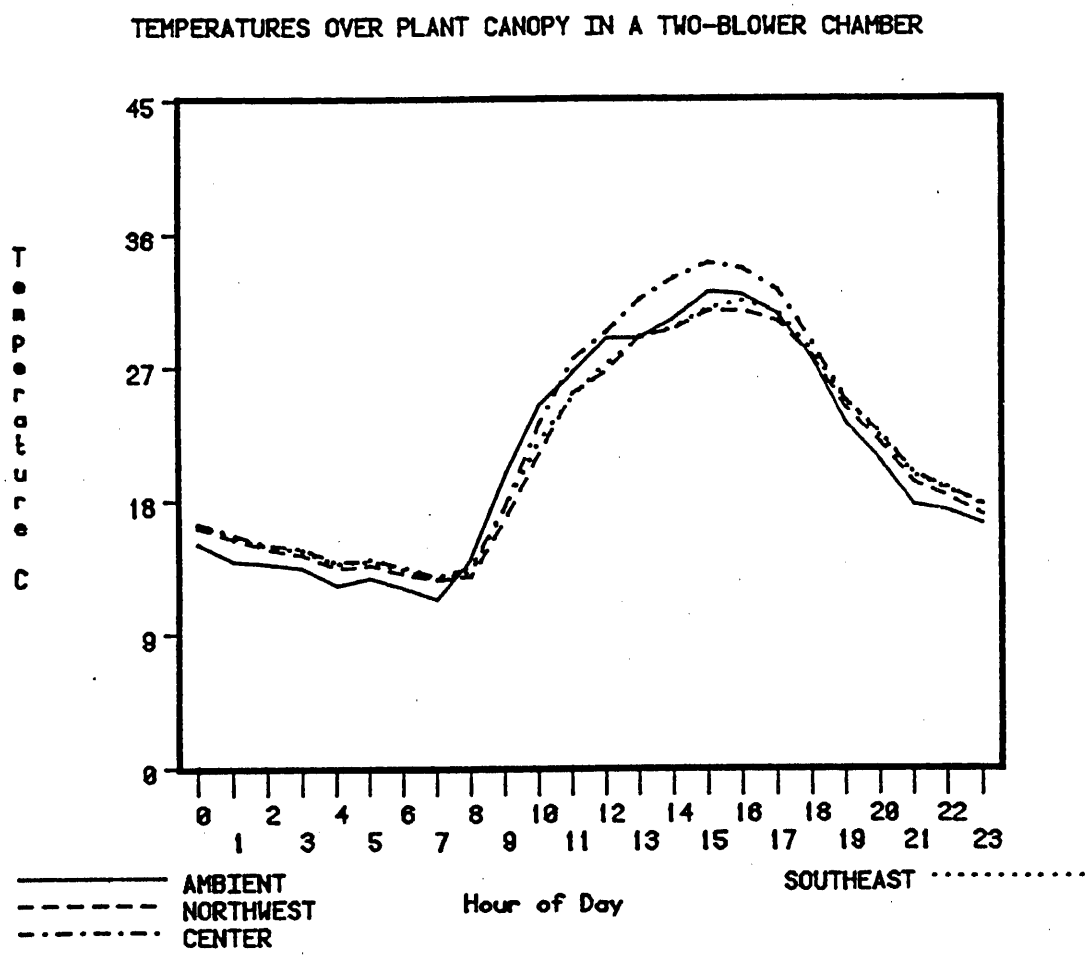

Figure I.7 Temperature variability within a two-blower chamber at 50 cm height in the presence of a developed plant canopy.

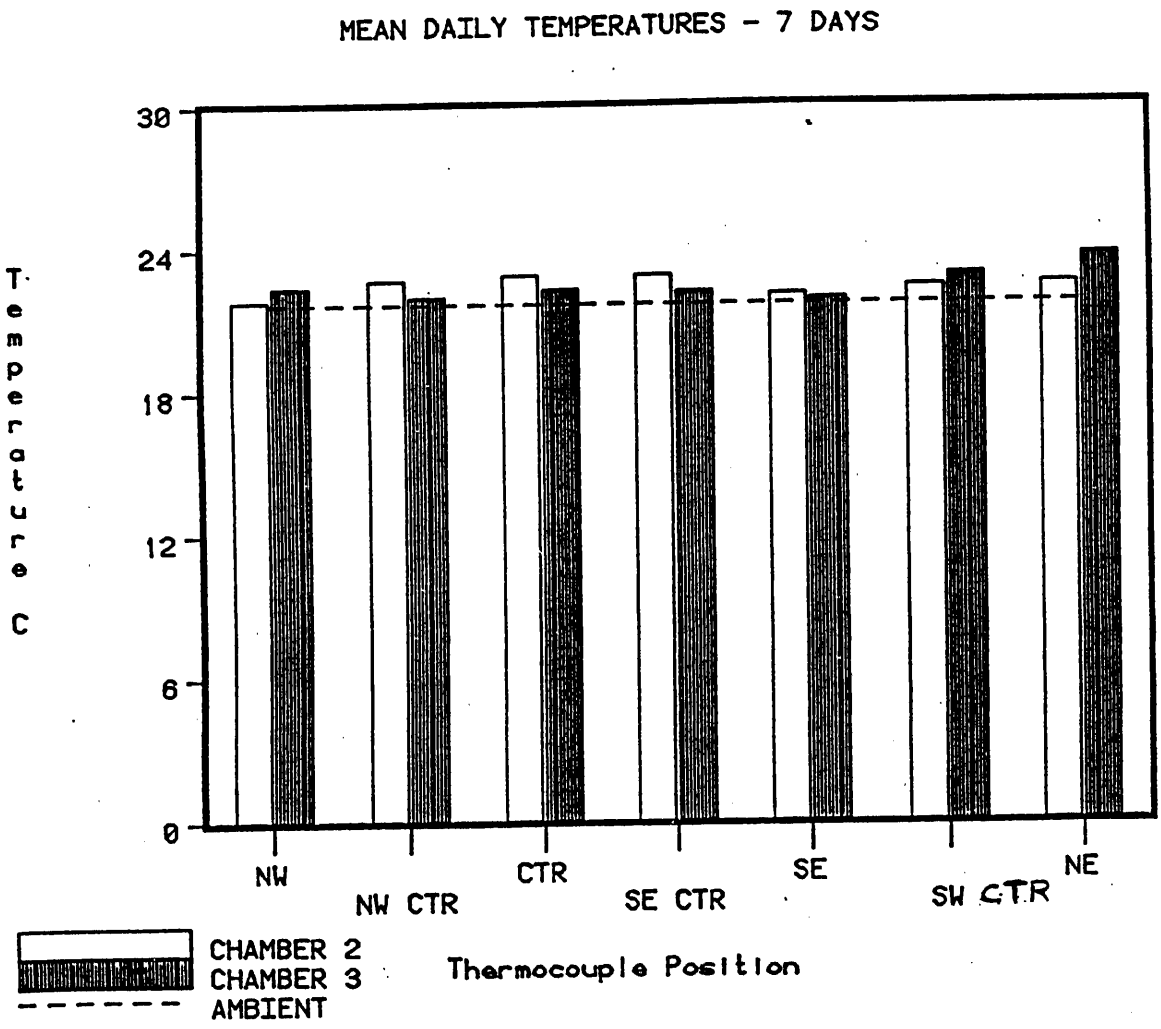

Figure I.8 Variability of mean 24 hr temperatures over seven days within two chambers compared to ambient conditions.

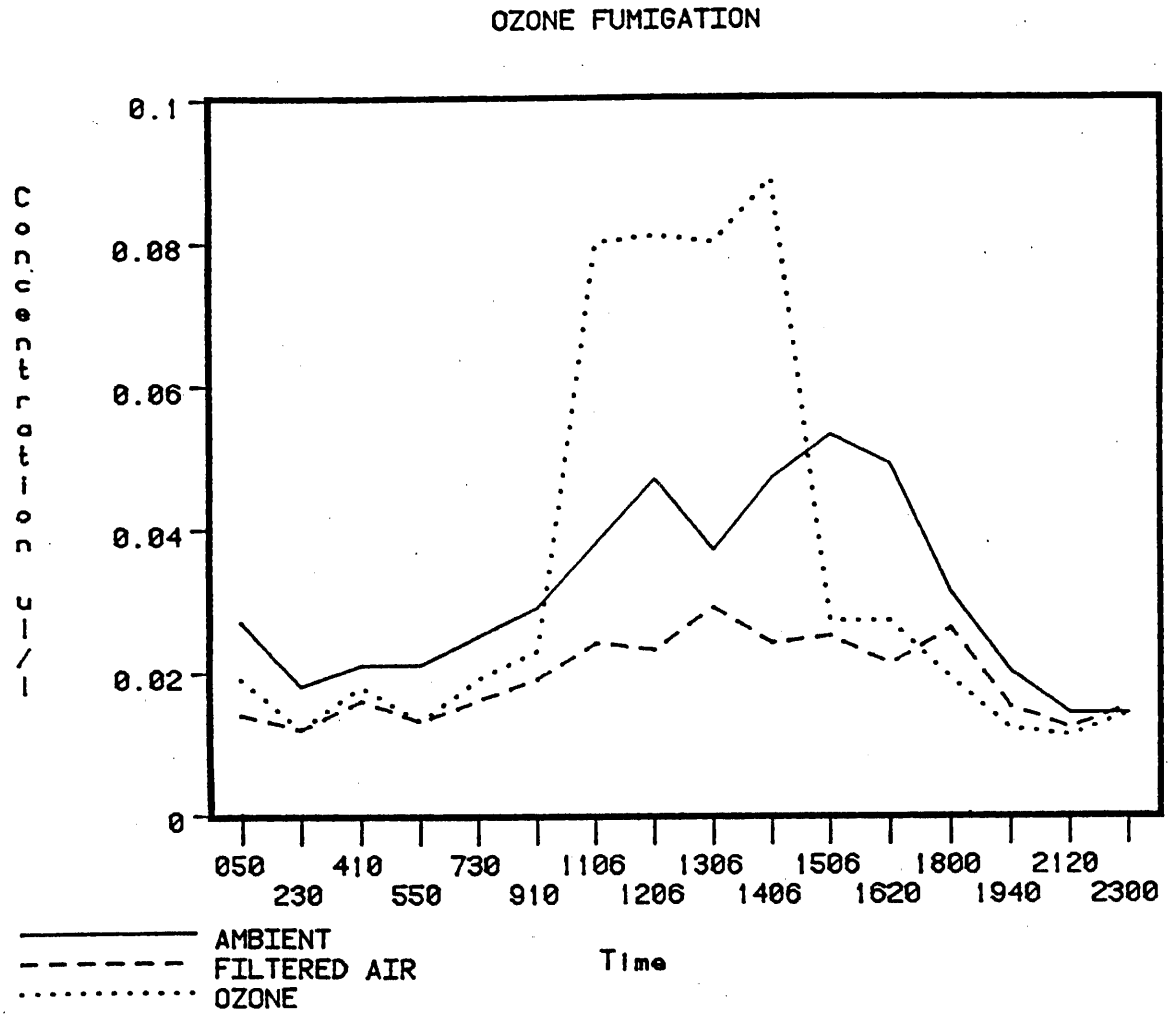

Figure I.9 Typical ozone concentrations during the experimental period in a fumigated chamber, a chamber receiving filtered air and ambient conditions.

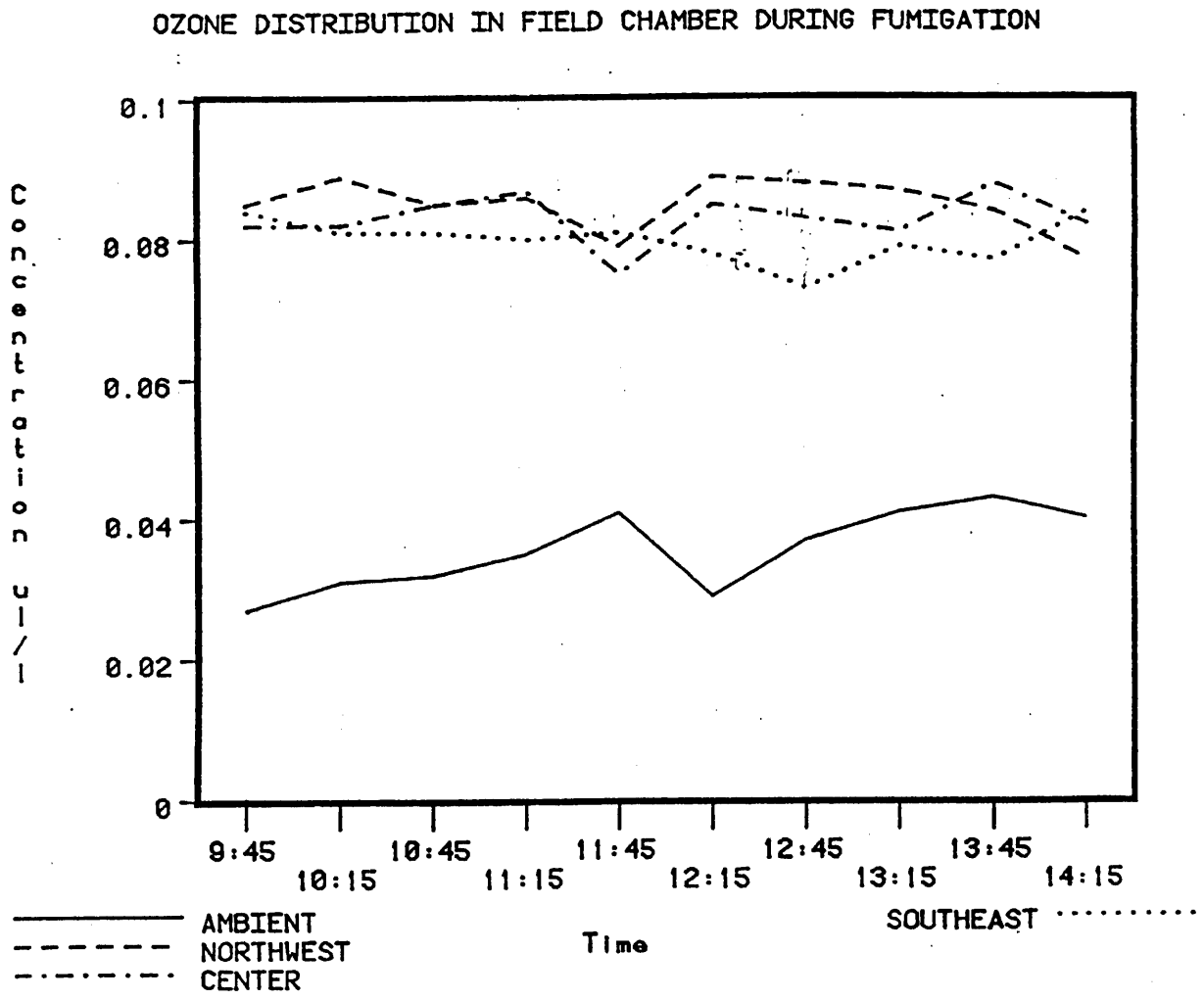

Figure I.10 Ozone concentration variability within a fumigated chamber during an exposure period, in comparison with ambient conditions.

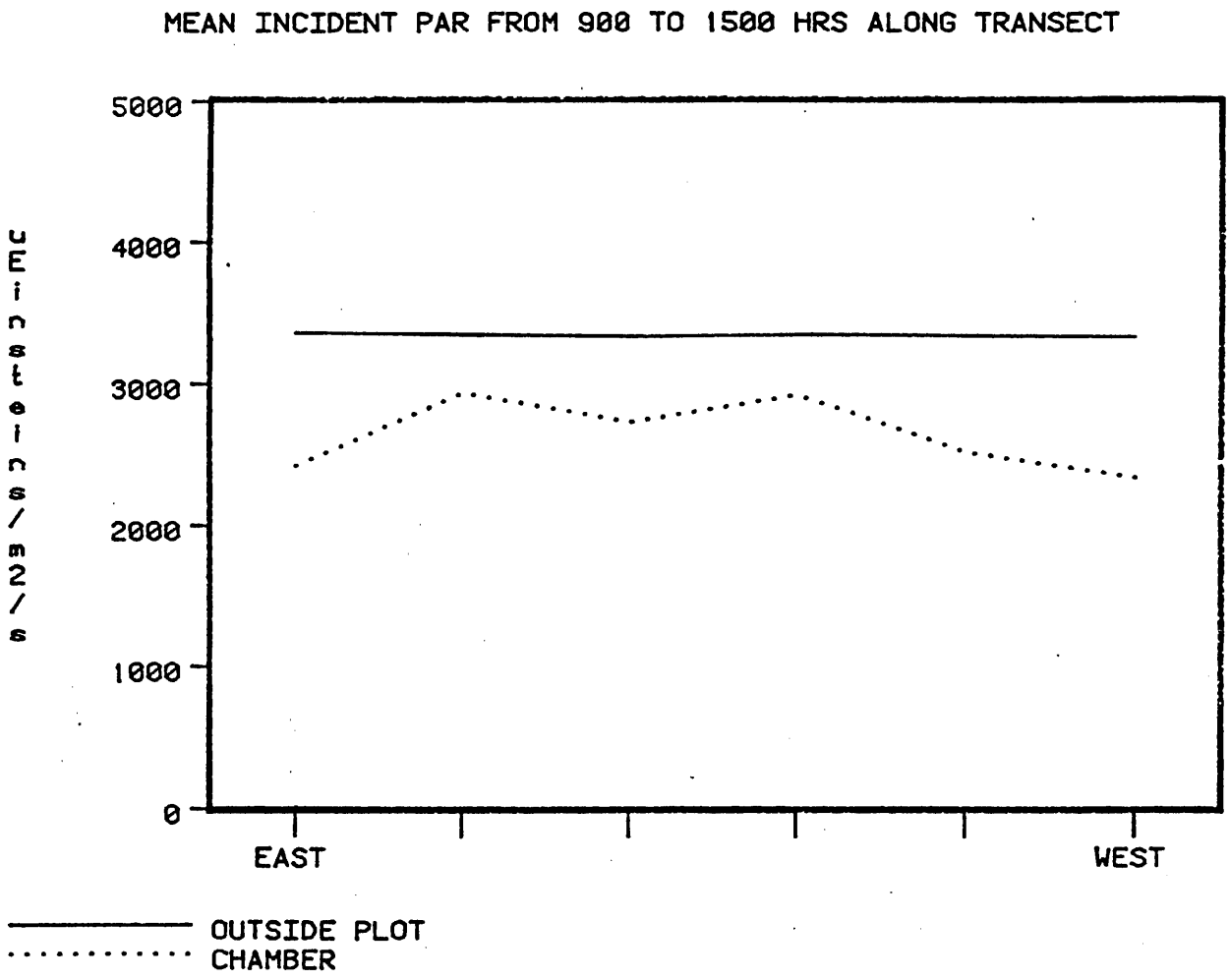

Figure I.11 Mean incident photosynthetically active radiation (PAR, 400-700 nm) along transects in a chamber and an outside plot from 0900 to 1500 hrs.

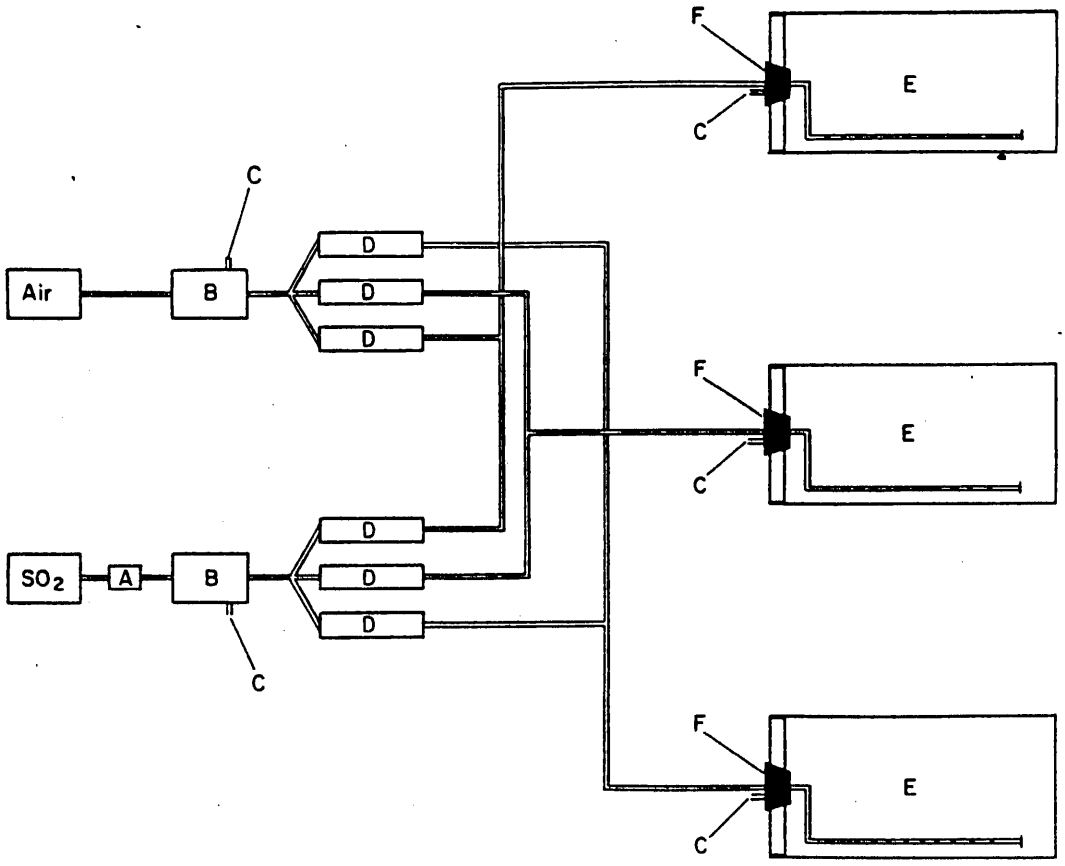

Figure I.12 Schematic diagram showing gas delivery system and three fumigation chambers. A = in-line low pressure regulator; B = scrubbing tower; C = exhaust port to outdoors; D = glass capillary tubes chosen to deliver desired gas flow; E = glass exposure chambers with perforated Tygon tubing for more uniform gas delivery within chamber; F = sampling port and sampling tube.

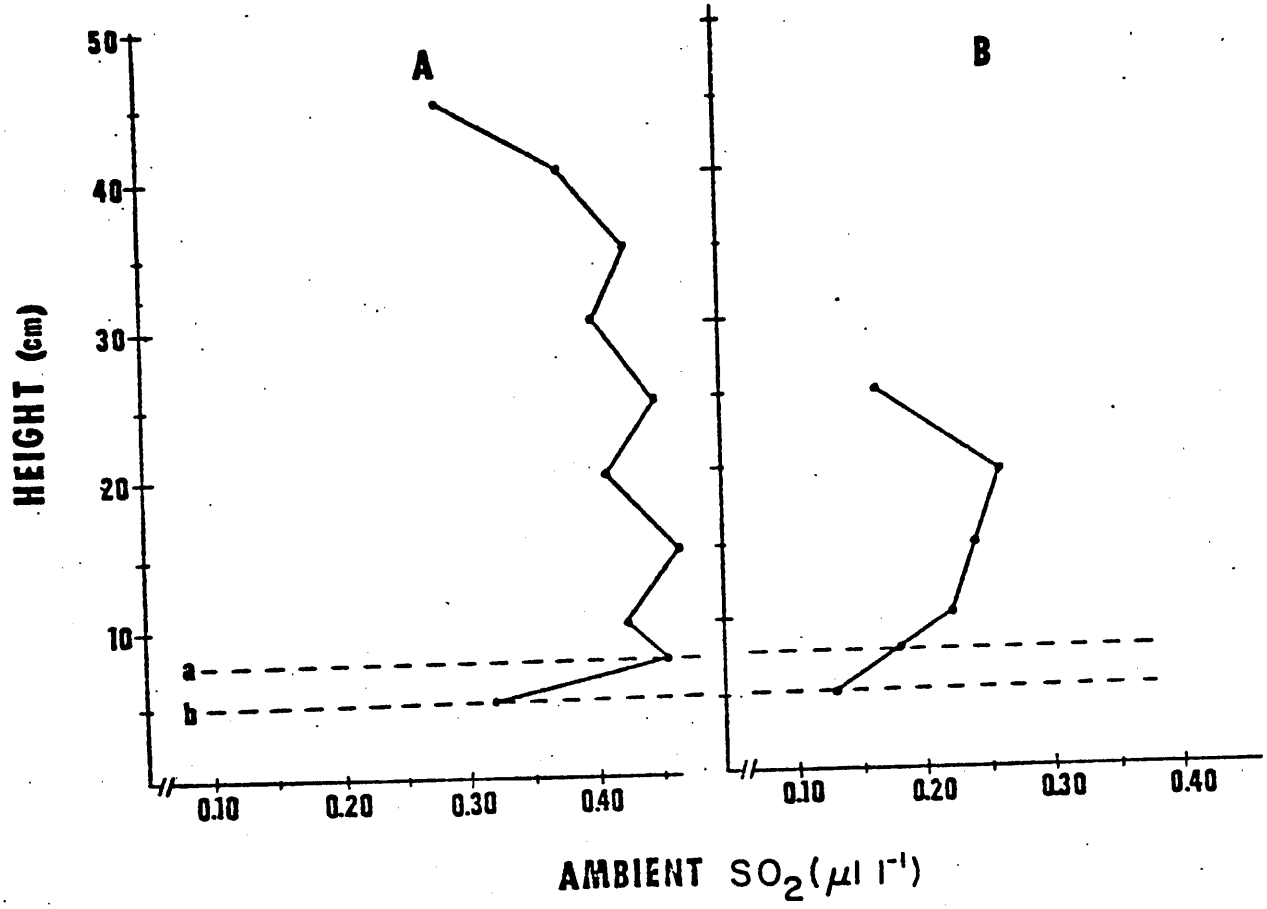

Figure I.13 Concentration gradient of ambient SO_2 in glass chamber, A (16 ℓ capacity) and B (9 ℓ capacity), fumigated with 0.75 μℓ ℓ^{-1} SO_2 at point (a), 3 cm above moistened vermiculite (b).

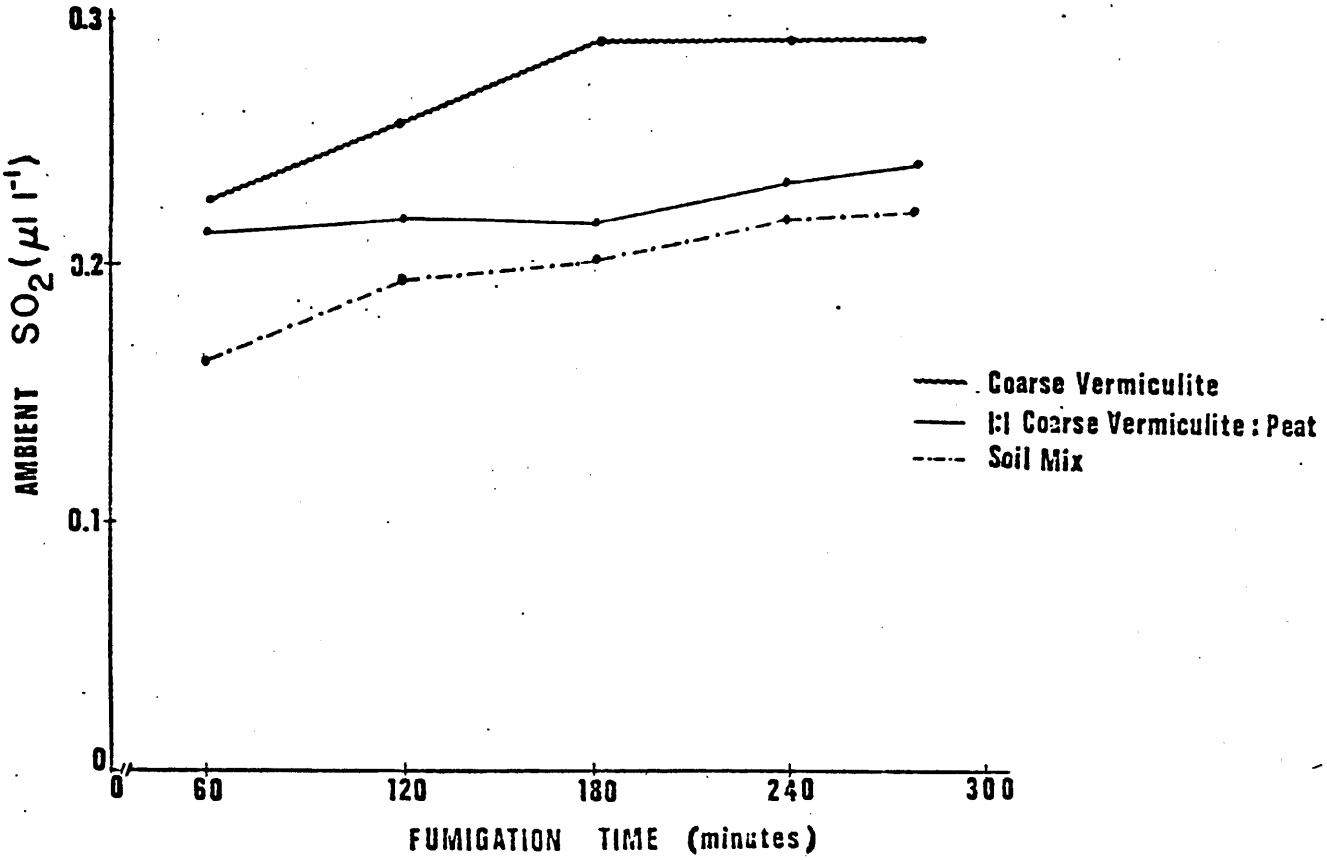

Figure I.14 Ambient SO_2 above three seed germinating media, wetted to field capacity, during four-hour fumigation with 0.75 $\mu\ell\ \ell^{-1}$ SO_2. Soil mix contained vermiculite:peat:sand on a v:v:v basis. Water holding capacity of each media is inversely correlated to ambient SO_2 concentration.

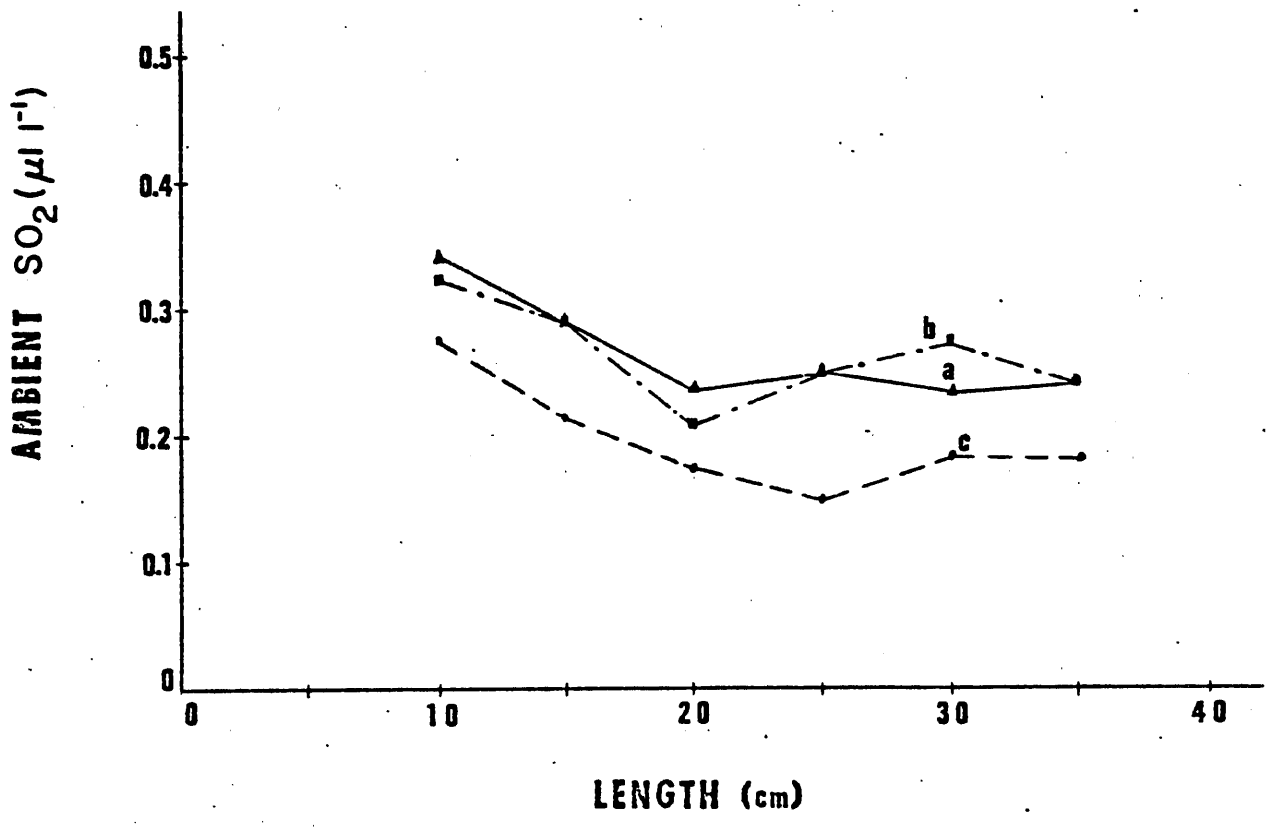

Figure I.15 Ambient SO_2 concentration along a horizontal transect above wetted vermiculite held in three different (a, b, c) 16 ℓ glass chambers after two hours of fumigation with 0.75 $\mu\ell\ \ell^{-1}$ SO_2.

CHAPTER II. Penetration and Oxidation of Sulfur Dioxide in Soil

1. Introduction

Sulfur dioxide is an important air pollutant which causes much damage to plants. The main route of entry of sulfur dioxide into the plant is through the leaf stomata, though conceivably sulfur dioxide deposited on the soil could enter the plant via the roots. Sulfur dioxide is rapidly absorbed by soil which has a large capacity for absorption (Smith et al., 1973; Yee et al., 1975). However, no information is available regarding the depth of penetration of sulfur dioxide into the soil. Sulfite, the hydrated form of sulfur dioxide, can be oxidized in soil to sulfate which is relatively nontoxic to plants. Faller and Herwig (1969) compared different soils for their ability to oxidize SO_2 to sulfate and found that more oxidation occurred in calcareous soils than in soils with low pH. Ghiorse and Alexander (1976) examined the fate of sulfur dioxide in a loam soil using relatively high concentrations (2,000-6,000 $\mu\ell\ \ell^{-1}$). They observed that for their shortest incubation time, ten hours, all of the sulfite was lost and about 75% of the sulfur was recovered as sulfate. We wanted to extend this work using radiolabeled sulfur dioxide which would enable us to use low levels of sulfur dioxide for short incubation periods, determine both sulfite and sulfate, and determine the penetration of sulfur dioxide in the soil.

2. Materials and Methods

Three soils were used, Yolo silty loam (pH 7.3, field water capacity 23.4%), Capay silty clay (pH 7.5, field water capacity 31.8%) and Metz variant fine sandy loam (pH 7.2, field water capacity 13.8%) and collected from the surface layer (0 to 15 cm) of soil from the University of California farm at Davis. the soils were crushed and passed through a 1.5 mm screen before using.

[^{35}S]Sulfur dioxide gas (Amersham-Searle) was absorbed in 0.15 N KOH which also contained 25% (v/v) ethanol and 3 mol m^{-3} EDTA. Ethanol and EDTA were included to inhibit autooxidation.

To determine penetration of [^{35}S]sulfur dioxide into the soil, 5 g of soil was placed into a 12 ml plastic syringe which had the tip

end removed. The syringe was sealed using a serum stopper with attached center well containing [^{35}S]sulfite (15,100 Bq; 36 nmol). With a hypodermic syringe and needle, 2 N H_2SO_4 was added to the center well to liberate sulfur dioxide gas. After 15 minutes the serum stopper and center well were removed and the top of the syringe was cut off just above the soil surface. The plunger of the syringe was carefully pushed up to expose 2 mm of soil which was scraped off into a test tube containing 0.5 ml 0.04 M tetrachloromercurate (Scaringelli et al., 1967). This was repeated until soil to a depth of 1 to 2 cm was removed. Then the radioactivity in the tetrachloromercurate solution was determined by scintillation counting.

To measure oxidation of [^{35}S]sulfur dioxide in soil, one g of soil was placed in a 25 ml glass scintillation vial which gave a surface area and depth of 4.2 cm^2 and 1 to 2 mm, respectively. [^{35}S]Sulfur dioxide was generated in a 5 ml shell vial by adding H_2SO_4 to potassium [^{35}S]sulfite (83,000 Bq; 45 nmol). The generated gas was then transferred via tubing to the scintillation vial with soil which had been evacuated. After 15 or 30 minutes, the vial was opened and the soil transferred to a tube containing 2 ml 0.04 M tetrachloromercurate, shaken and allowed to stand about 30 minutes. Then paper electrophoresis at pH 7 was performed on an aliquot of this solution and the ratio of [^{35}S]sulfite/[^{35}S]sulfate was determined using a radioscanner.

3. Results

In relation to the fate and metabolism of sulfur dioxide in soil, we wanted to know the depth of penetration of sulfur dioxide into the soil. We observed in both the Yolo silty loam and the Metz sandy loam that penetration was very shallow with only 5% or less of the sulfur dioxide penetrating more than 2 mm below the surface and no sulfur dioxide was found below 1 cm (Table II.1). For technical reasons it was necessary to use soil of small particle size (passed through 1.5 mm screen). Therefore with larger soil particle size as in the field, penetration would probably be greater than what we observed, yet we expect that most of the sulfur dioxide would be absorbed in the top surface of the soil.

Oxidation of [^{35}S]sulfur dioxide was rapid in all three soils (Table II.2). Comparing the soils in the air-dry condition, oxidation was fastest in the Metz sandy loam, followed by the Capay silty clay and then the Yolo silty loam. With a soil moisture content of 55% field capacity, oxidation was more rapid than in the air-dry condition for each soil and little difference in oxidation rate was apparent between the three soils. It is difficult to determine from our data whether actual oxidation is faster in a moist than a dry soil or that this is simply a result of enhanced absorption which has been observed for moist soils (Smith et al., 1973; Yee et al., 1975). We observed ^{35}S only as [^{35}S]sulfite and [^{35}S]sulfate. No other products were detectable and nearly all (98 to 99.5%) of the ^{35}S in the soil was extracted with the tetrachloromercurate solution.

These results are supported by work done in the 16-liter chambers (Chapter I) with germinating seeds in various soil media. SO_2 (0.75 $\mu\ell\ \ell^{-1}$) flowed into the chambers and the ambient concentration within the chambers was measured. As Figure II.1 illustrates, the absorption of SO_2 by moist vermiculite is twice the amount absorbed by the dry material. Further work with different potting media (coarse vermiculite, coarse vermiculite:peat 1:1, and a commercial potting mix) indicated the absorption of SO_2 by the medium was inversely correlated to water holding capacity. Bean seeds germinated in these media with ambient SO_2 concentrations ranging from 0.2 to 0.3 $\mu\ell\ \ell^{-1}$ showed no change in germination or root growth compared to the controls. The experiment was terminated at seedling emergence.

4. Discussion

In our soil experiments, sulfur dioxide concentration was 30 to 35 $\mu\ell\ \ell^{-1}$ or about 2.2 µg sulfur dioxide. To relate this static concentration to a more realistic situation, that of a flow system, we have used the data of Lockyer et al. (1978). Using their data one can calculate that with a sulfur dioxide concentration of approximately 0.05 $\mu\ell\ \ell^{-1}$ (144 $\mu g\ M^{-3}$), flow rate of 100 $cm^3 s^{-1}$ and sorption velocity of 0.264 for an air dry soil, 2.2 µg of sulfur dioxide would be absorbed in approximately 20 minutes.

Sulfite can be oxidized to sulfate through either an ionic or a free radical mechanism (Schroeter, 1966). Generally the ionic mechanism predominates at high concentrations (M range) and the free radical mechanism at low concentrations (mM range). The free radical mechanism has been carefully studied in aqueous solution and it can be initiated by uv light (Hayon et al., 1972) and transition metals such as manganese (Yang, 1970). The oxidation of sulfur dioxide in aerosols has recently been actively investigated, and in aerosols containing impurities oxidation via a free radical mechanism appears to be very important (Barrie and Gorgii, 1976; Beilke and Gravenhorst, 1978; Clarke and Williams, 1983). Oxidation of sulfur dioxide in aerosols was greatly enhanced with the addition of Mn^{2+} and Fe^{2+} (Barrie and Gorgii, 1976), and with $MgCl_2$, NaCl and $(NH_4)_2SO_4$ (Clarke and Williams, 1983). We attempted to examine the role free radicals might play in the oxidation of sulfite in soil using the free radical scavengers hydroquinone, tiron (1,2-dihydroxybenzene-3,5-dusulfonic acid), butylated hydroxytoluene and ethanol. Inhibition of sulfate formation was observed with each inhibitor, and hydroquinone and tiron were the most effective ones. However, these results are inconclusive since paper electrophoresis indicated that hydroquinone and tiron, or decomposition products of these compounds, were reacting with sulfite such that little to no free sulfite remained. Also high concentrations of these inhibitors were needed to prevent sulfate formation.

In conclusion our results indicate that sulfur dioxide penetrates only a very short distance into the soil and is rapidly oxidized to the nontoxic form, sulfate. This suggests that under most conditions, the deposition of sulfur dioxide on agricultural soils will have little adverse effect on most plants. Concern about injury to germinating seeds seems unjustified. None of our work has shown an adverse effect of low levels of SO_2 on germination (Chapters III and IV), and root penetration even by shallow seeded species should be deep enough to protect roots from exposure to SO_2 and sulfite.

5. Summary

Soils were exposed to low levels of radiolabeled sulfur dioxide ($^{35}SO_2$) for short incubation periods. Depth of penetration of sulfur dioxide into the soil was measured as well as rate of oxidation to sulfite and sulfate with no other products being detectable. Depth of penetration in all soils was very shallow; 95% of the sulfur dioxide remained within 2 mm of the surface with none penetrating below 1 cm. Oxidation was most rapid in the moist soils; over 90% was oxidized within 30 minutes. In the air dry soils 70% was oxidized within 30 minutes although actual absorption of sulfur dioxide by the dry soils is probably less. Exposure of potting media to a continuous flow of 0.75 $\mu\ell\ \ell^{-1}$ sulfur dioxide indicated a 50% reduction in absorption by the dry medium compared to the moist.

Seed germination and root growth of beans in potting media showed no adverse effects from six hours daily exposure to 0.25 $\mu\ell\ \ell^{-1}$ ambient SO_2, with the experiment terminated at seedling emergence. The conclusion is that under most conditions deposition of sulfur dioxide on agricultural soils will have little adverse effect on germination and root growth of plants.

6. References

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Table II.1 Penetration of sulfur dioxide into soil.

Depth (mm)	Radioactivity absorbed (%)	
	Yolo silty loam	Metz sandy loam
0 - 2	96	95
2 - 4	3	3
4 - 6	1	1
6 - 8	0	1
8 - 10	0	0

Table II.2 Oxidation of [^{35}S]sulfur dioxide in three soils at air dry and 55% field capacity (FC) moisture content. Soil was exposed to approximately 30 $\mu\ell^{-1}$ (2.2 μg) of [^{35}S]sulfur dioxide.

Soil moisture	Incubation time (min)	Oxidation (%)		
		Metz sandy loam	Yolo silty loam	Capay silty clay
Air dry	15	74	55	60
	30	93	70	76
55% FC	15	80	87	84
	30	96	96	93

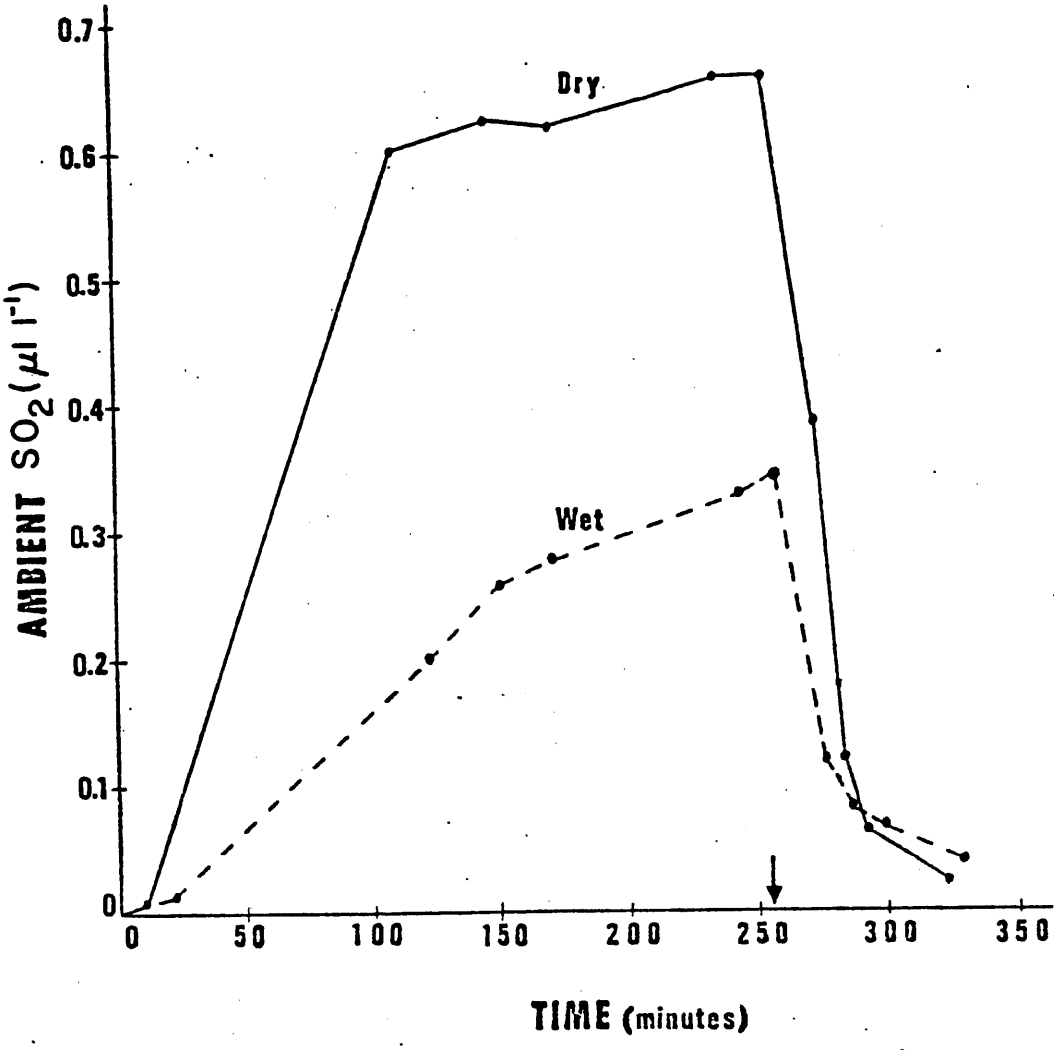

Figure II.1 Ambient SO_2 measured in two 16 ℓ glass chambers, one with wetted and the other with dry vermiculite, fumigated with 0.75 $\mu\ell\ \ell^{-1}$ SO_2. Arrow denotes cessation of fumigation.

CHAPTER III. Comparative Development: Tomato and Cucurbit

1. Introduction

Studies on the effects of air pollutants on the growth and physiology of plants have shown clearly that cultivars of the same species as well as species vary in their sensitivity to SO_2 and O_3 (Henderson and Reinert, 1979; Butler and Tibbits, 1979). The immediate goal of this project was to study selected physiological and biochemical processes under controlled environmental conditions in order to establish a foundation for determining how physiological responses to air pollutants are related to growth and yield. One of the tools for selecting pertinent physiological and biochemical processes was to study cultivars of the same species and different species and examine differential responses under fumigant stress. A permutation of this was to examine cultivars at different stages of development to determine if differential responses remained consistent throughout the life cycle of the plant, in spite of the changing roles of specific physiological and biochemical processes. Inconsistencies would indicate a specific, independent process conferring resistance or susceptibility; consistency would indicate a general, interdependent process. Chapter IV discusses our research results using corn varieties in the seedling and later vegetative stage. The physiological process closely examined in that study is stomatal behavior since experiments with seedlings were completed before stomatal complexes had developed and older plants have well-developed stomatal complexes. This chapter examines more diverse developmental stages: pollen and seedlings. Our work with corn had indicated a general response, and we hoped to extend that generalized response to these more diverse stages as well as to establish an efficient means of screening for varietal and species susceptibility to low levels of pollutants.

2. Materials and Methods

The experiments described in this chapter were conducted using the series of 16-liter glass chambers described in Chapter 1B.

Seed Germination and Seedling Growth

Seed germination and growth took place on a filter paper medium. After four hours imbibition in distilled water, seeds were placed on a filter paper shelf sandwiched between two pieces of filter paper backed by a Plexiglas plate. Wetting the papers kept them in position so the entire plate could stand in a dish containing a reservoir of either distilled water or MES (2 N-morpholino ethane sulfonic acid) buffer, pH 6.4. Molarity of the buffer was 0.005, enough to provide some buffering capacity with a minimum of inhibition to the developing seed. The liquid reservoir allowed the filter papers to remain wet throughout seed germination and seedling growth.

Two cultivars of processing tomato (VF 134 and VF 145) and three cultivars of cucurbits (National Pickling Cucumber, Eastern Prolific Straightneck Squash, and Sugar Pie Pumpkin) were tested. Ten to 20 seeds (dependent on how many fit on one filter paper plate) of each cultivar were placed in each of the 12 chambers. When SO_2 was applied, four levels of SO_2 (0, 0.25, .0.65, and 1.7 $\mu\ell\ \ell^{-1}$) were replicated three times. When fumigation with SO_2 and O_3 was tested, three levels of SO_2 (0, 0.25, and 1.0 $\mu\ell\ \ell^{-1}$) and two levels of O_3 (0 and 0.14 $\mu\ell\ \ell^{-1}$) were replicated two times. Fumigations began once the seeds were placed on the filter paper and put in the chambers; they continued for four hours daily until seedling cotyledons were fully expanded (6-8 days depending on species). Experiments were repeated a minimum of three times. Chambers were located in a constant temperature room of 25°C.

With three dishes in each chamber, gas flow rates were adjusted to give desired ambient concentrations. As discussed in Chapter 1B, ambient SO_2 concentration in the chambers is highly dependent on the surface area and water holding capacity of the media. The concentration of SO_2 delivered and the consequent ambient concentration in the chamber containing three germination plates was as follows:

delivered (SO_2 $\mu\ell\ \ell^{-1}$)	range in chamber (SO_2 $\mu\ell\ \ell^{-1}$)
1.60	0.25 - 0.50
2.75	0.65 - 1.10
6.00	1.7 - 2.6.

When cotyledons were fully expanded, the experiment was terminated. Plants were removed from the filter paper, root lengths and fresh weights were determined. The pH of each filter paper was measured using a pH-electrode.

Pollen Germination and Pollen Tube Growth

It was necessary to first develop and test pollen germination media. Basic ingredients and concentrations tested included sucrose, H_3BO_3, KNO_3, $MgSO_4 \cdot 7H_2O$, and $Ca(NO_3)_2 \cdot 4H_2O$ or $Ca(H_2PO_4)_2$. A range of media pH was evaluated. The basic ingredients of media were based upon existing reported literature (Kwack, 1964). Preliminary pollen germination tests were conducted by varying concentrations of ingredients in sterilized bacto-agar. The following ingredients and concentrations (brought to 100 ml volume) were tested:

Sucrose	(gm)	5	10	20	
H_3BO_3	(mg)	Ø	10	20	
KNO_3	(mg)	Ø	10	20	
$MgSO_4 \cdot 7H_2O$	(mg)	Ø	20	40	
$Ca(NO_3) \cdot 4H_2O$	(mg)	Ø	15	30	60
$Ca(H_2PO_4)_2$	(mg)	Ø	15	30	60
pH		6.0	7.0	8.0	

Reasonable permutations were carried out for each species of alfalfa, tomato, and cucumber pollen until a combination was attained that gave satisfactory pollen germination and tube growth consistently.

$Ca(NO_3)_2 \cdot 4H_2O$ was found to be the best source of Ca. The most vigorous tomato pollen tube growth occurred on a media without additional Ca, with decreasing germination occurring with increasing Ca concentration.

Media pH was found to have a marked influence on pollen germination and tube growth. Since media pH was affected by SO_2 fumigation, means of improving the buffering capacity were tested. Phosphate, MES, and PIPES (piperazine-N, N^1-bis 2-ethane sulfonic acid) buffers were tested. The phosphate buffer was judged to be least inhibitory to germination and growth. Table III.1 shows the

media composition used in subsequent SO_2 fumigations on alfalfa (a standard cultivar), cucumber (SMR 58), and tomato (VF 145) pollen.

Pollen was collected from both greenhouse and field grown plants. Freshly collected pollen was dusted over the agar medium surface and immediately placed into a chamber and fumigated for two hours at three levels of SO_2: 0.4 ± 0.07, 0.6 ± 0.1, and 1.0 ± 0.2 $\mu\ell\ \ell^{-1}$. A control, air only, was included. After removal from the chambers, plates were incubated at room temperature and 90% relative humidity for two hours. At that time the addition of a methyl green and phyloxin solution stopped growth and stained pollen grains and tubes. Pollen germination and tube growth was measured from photographs taken of the experimental material. Experiments were conducted at least five times.

3. Results

Seed Germination and Seedling Growth

Seed germination was not affected by fumigation with low levels of SO_2 or O_3. Seedling growth, however, was affected. Experiments exposing seedlings to SO_2 alone were completed using both distilled water (unbuffered) and the buffered medium. The tomato cultivars were very sensitive to low levels of SO_2 with no difference in cultivar response. Root elongation of both tomato cultivars was significantly reduced at 0.3 $\mu\ell\ \ell^{-1}$ SO_2 on both the unbuffered and buffered medium (Figure III.1). Considering treatment effect as a percent of the control (Table III.2), the two media performed about equally. At 0.5 $\mu\ell\ \ell^{-1}$ SO_2, tomato root growth was reduced 70% of the control; at 0.9 $\mu\ell\ \ell^{-1}$, reduction was near 90%.

Experiments exposing seedlings to SO_2 and O_3 in order to observe the interaction of SO_2 and O_3 were completed using only the MES-buffered medium. In tomato, there was a statistically significant interaction between SO_2 and O_3. Ozone at 0.14 $\mu\ell\ \ell^{-1}$ slightly but significantly decreased root elongation compared to the control. This reduction was not apparent or significant when SO_2 was present. It appears that SO_2 so severely affects the tomato root, that an addition of ozone is not a significant factor. This is borne out by using a factorial analysis and partitioning the treatment sum of squares into

main effects and interactions (Little, 1981). In the tomato data, 95% of the response variability was attributable to SO_2, 2% to O_3, and 2% to the SO_2 x O_2 interaction.

The cucurbits were not as sensitive to SO_2 as were the tomato cultivars, and the variability between seedlings was considerable (Figure III.2). Cucumber roots grown in the distilled water were unaffected by up to 2.0 $\mu\ell\ \ell^{-1}$ SO_2; cucumber roots in the MES-buffer were significantly decreased at 0.3 $\mu\ell\ \ell^{-1}$ SO_2 (Figure III.2). Squash roots in distilled water were significantly reduced at 0.9 $\mu\ell\ \ell^{-1}$ SO_2; root reduction at 0.9 $\mu\ell\ \ell^{-1}$ SO_2 in MES-buffer was much greater (Table III.2). Pumpkin roots grown in distilled water were also significantly reduced at 0.9 $\mu\ell\ \ell^{-1}$ SO_2 (to 41% of the control), but the reduction in the MES-buffer was not as great (to only 16% of the control).

In the SO_2 x O_3 interaction experiments, neither the main factor ozone nor the SO_2 x O_3 interaction was significant. In the factorial analysis, 70% of the variability was attributable to species (e.g. there was a significant difference in root growth between cucumber, squash and pumpkin) and 20% was attributable to the main factor SO_2. The SO_2 factor was significant at the 1% level.

As mentioned earlier, the reason for using the MES-buffer was to limit a change in the media pH caused by the treatment. Table III.3 presents average pH of the filter paper in the root zone taken at termination of the experiments. The MES-buffer, even at 0.005 M, was effective at stabilizing pH over the range of SO_2 treatments. In the distilled water, the roots themselves influenced pH. With cucurbits, filter paper pH dropped from pH 6.6 to pH 5.6 in the presence of 1.5 $\mu\ell\ \ell^{-1}$ SO_2. With tomato, pH dropped from 6.4 to 3.9.

Because of the sensitivity of tomato cultivar VF 134 to SO_2, it was chosen to test possible interactive effects between concentration and time. The following five treatments were administered:

1) air only,
2) 0.3 $\mu\ell\ \ell^{-1}$ SO_2 four hours daily on days 1 and 2,
3) 0.3 $\mu\ell\ \ell^{-1}$ SO_2 four hours daily on days 3 and 4,
4) 0.3 $\mu\ell\ \ell^{-1}$ SO_2 four hours daily on days 1, 2, 3, and 4, and

5) 0.3 $\mu\ell\ \ell^{-1}$ SO_2 four hours daily on days 1, 2, 3, 4, 5, and 6.

All of the treatments were significantly different at the 5% level. The effect of SO_2 concentration and exposure time was additive (Figure III.3). Root growth response to SO_2 was dependent on the stage of seedling development. Seedlings fumigated the third and fourth days exhibited greater root retardation than those fumigated the first and second days. As the dose (concentration · hours) increased, root growth decreased.

Pollen Germination and Pollen Tube Growth

Media pH, pollen germination, and pollen tube growth were measured after exposure to two hours of SO_2. Two media were used each time, an unbuffered and a buffered. Figure III.4 shows the effect of two hours of SO_2 fumigation on the pH of the buffered and unbuffered media. The addition of the phosphate buffer effectively stabilizes pH even at SO_2 levels of 1.0 $\mu\ell\ \ell^{-1}$. The pH of the unbuffered media dropped from 6.2 in the control (no SO_2) to 4.5 after two hours of 1.0 $\mu\ell\ \ell^{-1}$ SO_2. There was little difference in media pH response to SO_2 between alfalfa, cucumber, or tomato.

Figure III.5 shows the effect of fumigation on percent pollen germination of alfalfa, cucumber and tomato grown on the buffered and unbuffered media. In all species, percent germination was decreased by adding the buffer to the media. In alfalfa and cucumber, SO_2 up to 1.0 $\mu\ell\ \ell^{-1}$ had no effect on pollen germination. In tomato, pollen germination on the unbuffered medium was significantly reduced at 0.4 $\mu\ell\ \ell^{-1}$ and again significantly reduced at 0.6 $\mu\ell\ \ell^{-1}$ with no further reduction occurring at 1.0 $\mu\ell\ \ell^{-1}$. However, since tomato pollen germination was affected by SO_2 only on the unbuffered medium, it is probable that the change in pH is the direct cause of the response rather than the SO_2.

The effect of the fumigation on growth of the pollen tubes is shown in Figure III.6. SO_2 affected tube growth of pollen in alfalfa, cucumber and tomato grown on both the buffered and unbuffered medium. Pollen tube growth on the buffered media was not as vigorous as growth on the unbuffered media. Furthermore, reduction in growth due to the SO_2 was less severe on the buffered media than on the unbuffered.

In alfalfa, average pollen tube length on the unbuffered media decreased from 2.5 mm to 0.6 mm with the addition of 0.4 $\mu\ell\ \ell^{-1}$ SO_2 for two hours. Reduction in tube growth on the buffered media was significant but much less severe (from 0.35 mm to 0.28 mm). Tube growth on the buffered media continued to decrease as SO_2 concentration increased even though pH remained stable. This indicates that SO_2, independent of its effect on pH change in the media, is influencing tube growth.

In cucumber, as in alfalfa and tomato, there was a marked decrease in tube growth in the control treatment of the buffered media compared to the control of the unbuffered media. At 0.4 $\mu\ell\ \ell^{-1}$ SO_2, tube growth on the unbuffered media was unaffected but on the buffered media it was significantly increased compared to the control. At 0.6 and 1.0 $\mu\ell\ \ell^{-1}$, tube growth significantly decreased on the unbuffered media. On the buffered media, however, while tube growth at 1.0 $\mu\ell\ \ell^{-1}$ was significantly less than growth at 0.6 $\mu\ell\ \ell^{-1}$, neither was significantly different from the control. This would suggest that change in pH is responsible for decreased tube growth on the unbuffered media while SO_2 on the buffered media with no change in pH actually enhanced tube growth at 0.4 $\mu\ell\ \ell^{-1}$ and had no effect on tube growth at higher levels.

Tomato pollen tube growth, like alfalfa, was very sensitive to change in media pH. In the unbuffered media, tube growth was reduced from 0.38 mm in the control to less than 0.1 mm at 0.4 $\mu\ell\ \ell^{-1}$. Growth was significantly reduced at the higher levels of SO_2. On the buffered media, tube growth was not significantly reduced until 0.6 $\mu\ell\ \ell^{-1}$ SO_2. Unlike the alfalfa and cucumber pollen, treatment with SO_2 on the buffered media less severely retarded tube growth than on unbuffered media. This suggests that tomato pollen tube growth is more sensitive to medium pH than to fumigation with SO_2.

4. Discussion

Exposure of both germinating seedlings and pollen to SO_2 highlight the interaction of SO_2 and medium pH. In both sets of experiments, use of buffered media changed the response to SO_2. At the seedling stage, use of buffered medium caused more severe root

reduction than use of unbuffered medium, probably because at the pH maintained by the buffer (6.4), the formation of HSO_3^- is favored (Spedding et al., 1980). Roots are more sensitive to HSO_3^- than to pH reduction. At the pollen stage, however, use of buffered media caused less significant reduction in tube growth compared to the control than use of the unbuffered medium. In fact, in cucumber, exposure to low levels of SO_2 using the buffered media actually enhanced tube growth. Pollen is more sensitive to pH reduction than to SO_2.

Both germinating seedlings and pollen were influenced significantly by exposure to low levels of SO_2. These results indicate some possibilities for using these plant materials to screen quickly and efficiently for sensitivity to pollutants. As discussed in Chapter IV, exposure of three varieties of corn seeds and seedlings to low levels of SO_2 produced significant differences in cultivar sensitivity that are consistent with differences found in older plants. Tomato seedlings were found to be extremely sensitive to SO_2, but whether this is also true of older tomato plants was not studied. Although the seedling experiments took longer to complete than the pollen experiments, the results were much more consistent. Pollen proved to be extremely variable not in the direction of response but in the magnitude of germination and growth. Many months were spent trying to improve the germination rate of tomato pollen, only to discover that the time of year was critical. Pollen must be harvested from the field or greenhouse in the late spring to produce a better than 70% germination rate. The only alternative is to produce pollen from plants grown in growth chambers simulating spring conditions. This would be a severe limitation in applying pollen response as a screening technique.

The comparison of the two stages (seed and seedling versus pollen) and their responses to low levels of SO_2 is interesting but inconclusive. Neither seed germination nor pollen germination was particularly affected by SO_2. Only tomato pollen on the unbuffered media showed a reduction in germination, probably due to the reduction in pH. Tomato seedlings showed an extreme sensitivity to SO_2 that was increased in the buffered medium. Cucumber seedlings were not nearly as sensitive and cucumber seed germination was unaffected by SO_2.

These same trends were repeated in the pollen experiments. Tomato pollen germination on the unbuffered media was negatively affected; cucumber germination was unaffected. Tomato pollen tube growth was reduced by SO_2 on both media; cucumber tube growth on the buffered media was actually enhanced at 0.4 $\mu\ell\ \ell^{-1}$ SO_2. The extreme difference between tomato and cucumber in the seedling experiments was not repeated in the pollen experiments, but the differences were there. These consistencies in response may be coincidental; many such consistencies are evidence that there is a general, interdependent process conferring variety and species susceptibility to pollutants.

5. Summary

This chapter discusses the results of exposing tomato and cucurbit seeds and seedlings and alfalfa, cucumber and tomato pollen to low levels of SO_2. The results of experiments of two such diverse developmental stages (seedling and pollen) are presented together in order to compare the responses. Consistencies in response would indicate a general, interdependent process conferring resistance to the pollutant rather than a specific physiological phenomenon (e.g. stomatal behavior).

Seeds of two cultivars of tomato and three cucurbit cultivars were exposed daily to four hours of various levels of SO_2 alone and SO_2 and O_3. The experiments lasted about one week or until seeds had germinated and the cotyledons were fully expanded. Percent germination and root length were measured. Pollen from alfalfa, cucumber (one of the cucurbits tested at the seedling stage), and tomato was dusted onto agar media and exposed for two hours to SO_2. Percent germination and pollen tube growth were measured. Due to the direct effect SO_2 has on media pH, all of the experiments were completed using both buffered and unbuffered media.

Seed germination and pollen germination were unaffected by SO_2. The only significant change in germination occurred in tomato pollen on the unbuffered medium. Tomato pollen was very sensitive to pH, and unbuffered medium pH in all of the experiments was dependent on SO_2 concentration. Root growth of tomato seedlings was extremely retarded by SO_2. Cucurbit roots were much less sensitive to SO_2 but were

significantly reduced by exposure to SO_2. Both tomato and cucurbit root growth was more negatively affected by SO_2 on the buffered medium than on the unbuffered. This is probably due to the formation of HSO_3^- which is favored at the pH (6.4) maintained by the buffer. It appears that roots are more sensitive to HSO_3^- than to a reduction in medium pH.

In the SO_2 and O_3 experiments on tomato seedlings, ozone alone significantly reduced root growth. In the presence of SO_2, however, there was no additional reduction, probably because damage by SO_2 was so severe. In cucurbits, neither ozone nor the SO_2 x ozone interaction was significant.

Tomato seedlings were used to study concentration and length of exposure interactions. Results indicated no significant interaction; concentration and length of exposure were additive. As the dose (concentration · hours) increased, root growth decreased.

SO_2 affected pollen tube growth of alfalfa, cucumber, and tomato grown on both the buffered and unbuffered medium. Generally tube growth decreased for all species with the addition of SO_2. The exception was cucumber on the buffered medium. At the lowest SO_2 level (0.4 $\mu\ell\ \ell^{-1}$) tube growth was enhanced. Pollen tube growth on the buffered medium seemed less affected by SO_2 than on the unbuffered medium, suggesting a greater sensitivity to medium pH than to SO_2.

6. References

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Table III.1 Pollen germination media composition for tomato, alfalfa and cucumber used in SO_2 fumigation tests.

Alfalfa	Tomato	Cucumber
0.001 M phosphate buffer	0.001 M phosphate buffer	0.001 M phosphate buffer
10 gm sucrose	10 gm sucrose	10 gm sucrose
10 mg H_3BO_3	10 mg H_3BO_3	10 mg H_3BO_3
20 mg $MgSO_4 \cdot 7H_2O$	20 mg $MgSO_4 \cdot 7H_2O$	20 mg $MgSO_4 \cdot 7H_2O$
10 mg KNO_3	10 mg KNO_3	30 mg Ca $(NO_3)_2$
15 mg Ca $(NO_3)_2$	1.5 gm agar	1.5 gm agar
1.5 gm agar		
Volume adjusted to 100 ml with either distilled H_2O (pH adjusted to 6.4*) or 0.005 M phosphate buffer.	Volume adjusted to 100 ml with either distilled H_2O (pH adjusted to 6.4*) or 0.005 M phosphate buffer.	Volume adjusted to 100 ml with either distilled H_2O (pH adjusted to 6.4*) or 0.005 M phosphate buffer.

*pH adjusted with 1 normal KOH.

Table III.2 Comparative root growth response to SO_2 fumigation of tomato and cucurbit seedlings germinated on distilled water and on MES-buffer medium (pH 6.4).

Cultivar	SO_2 ($\mu\ell\ \ell^{-1}$)	Percent change from control* H_2O	MES	Species	SO_2 ($\mu\ell\ \ell^{-1}$)	Percent change from control* H_2O	MES
VF 134	0.5 ± 0.1	-70a	-70a	Cucumber	0.3 ± 0.05	+4a	-32b
	0.9 ± 0.1	-90a	-85b		0.8 ± 0.1	+15a	-64b
VF 145	0.5 ± 0.1	-75a	-69b	Squash	0.03 ± 0.05	+29a	+4b
	0.9 ± 0.1	-92a	-88a		0.8 ± 0.1	-25a	-67b
				Pumpkin	0.3 ± 0.05	+6a	-8b
					0.8 ± 0.1	-41a	-16b

*Values followed by a common letter are not significantly different at the 5% level.

Statistical analysis based on Duncan's multiple range test after significance found with analysis of variance.

Table III.3 Comparison of pH of media in root zone of tomato and cucurbit seedlings germinated in distilled water and in MES-buffer medium (pH 6.4) after four hours daily fumigation for five days with SO_2 at 25°C.

TOMATO			CUCURBIT		
	pH			pH	
SO_2 ($\mu\ell\ \ell^{-1}$)	H_2O	MES	SO_2 ($\mu\ell\ \ell^{-1}$)	H_2O	MES
0	6.4	6.3	0	6.6	6.4
0.5 ± 0.1	5.2	6.2	0.3 ± 0.05	6.3	6.4
0.9 ± 0.1	4.5	6.2	0.8 ± 0.1	5.6	6.3
1.5 ± 0.2	3.9	6.2	1.5 ± 0.2	5.6	6.3

Statistical analysis based on Duncan's multiple range test after significance found with analysis of variance.

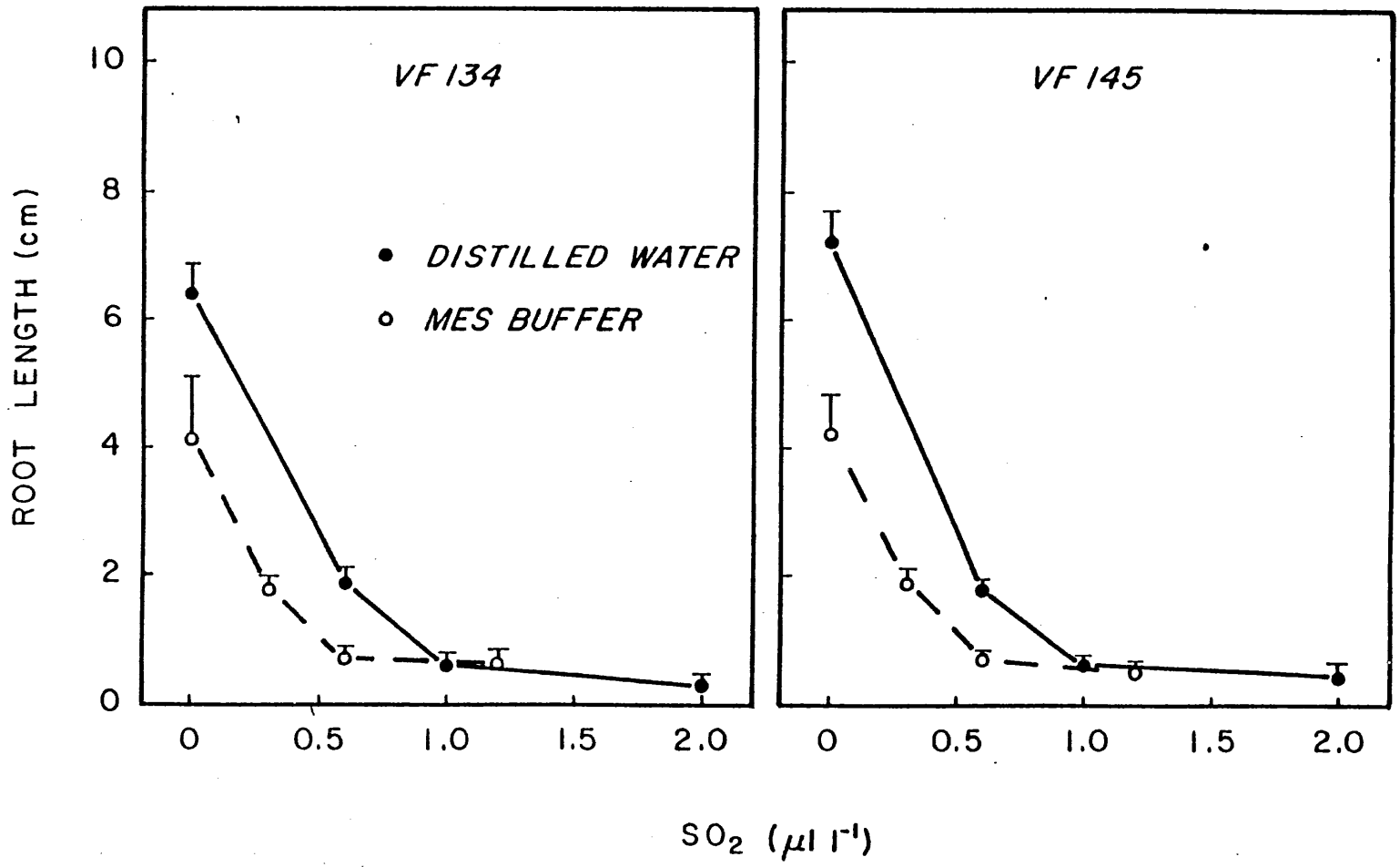

Figure III.1 The effect of four hour daily fumigation for six days at 25°C on root elongation of two cultivars of tomato seedlings grown in distilled water and in a MES-buffer medium.

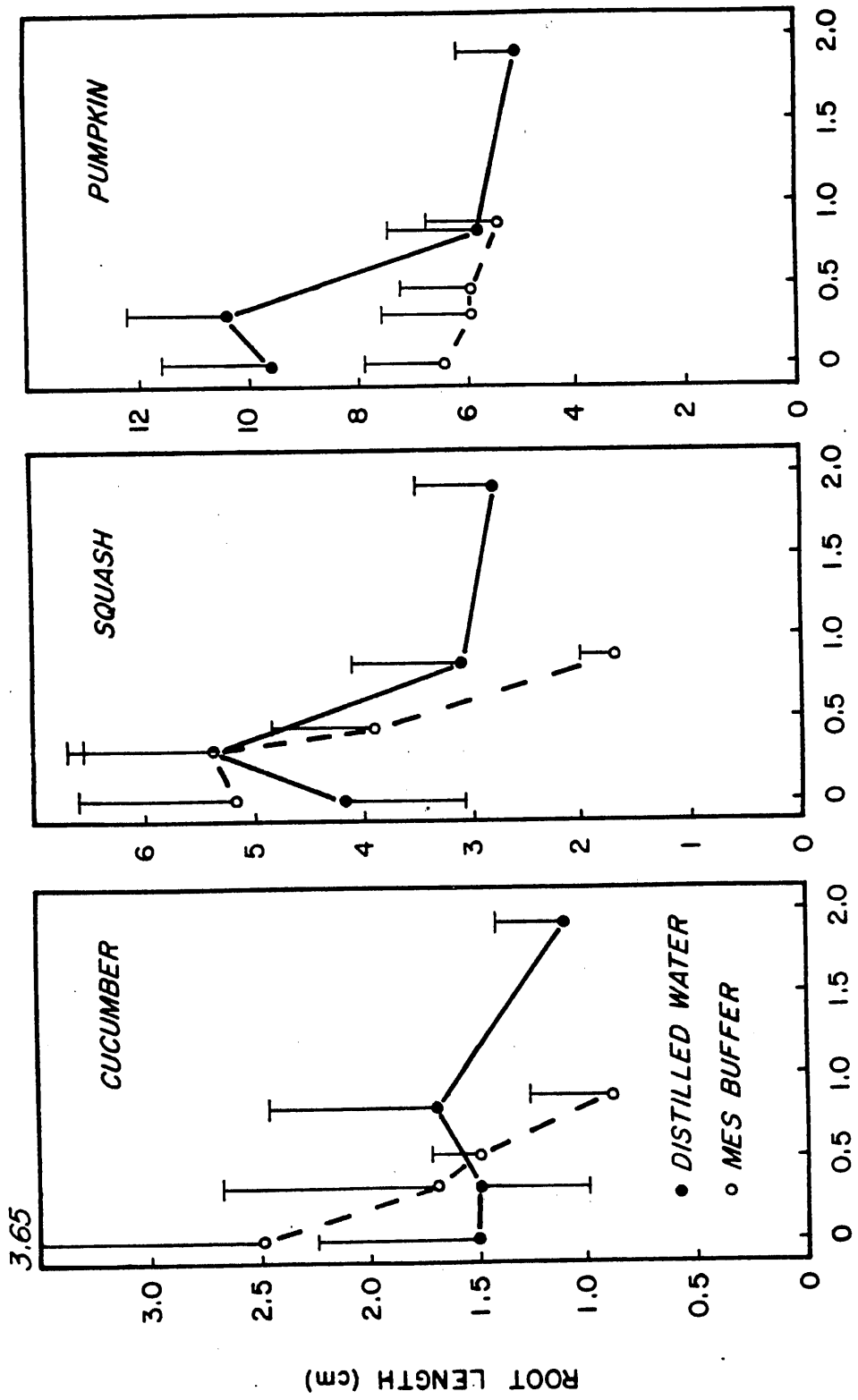

Figure III.2 The effect of four hour daily fumigation for five days at 25°C on root elongation of three species of cucurbit seedlings grown in distilled water and in a MES-buffer medium.

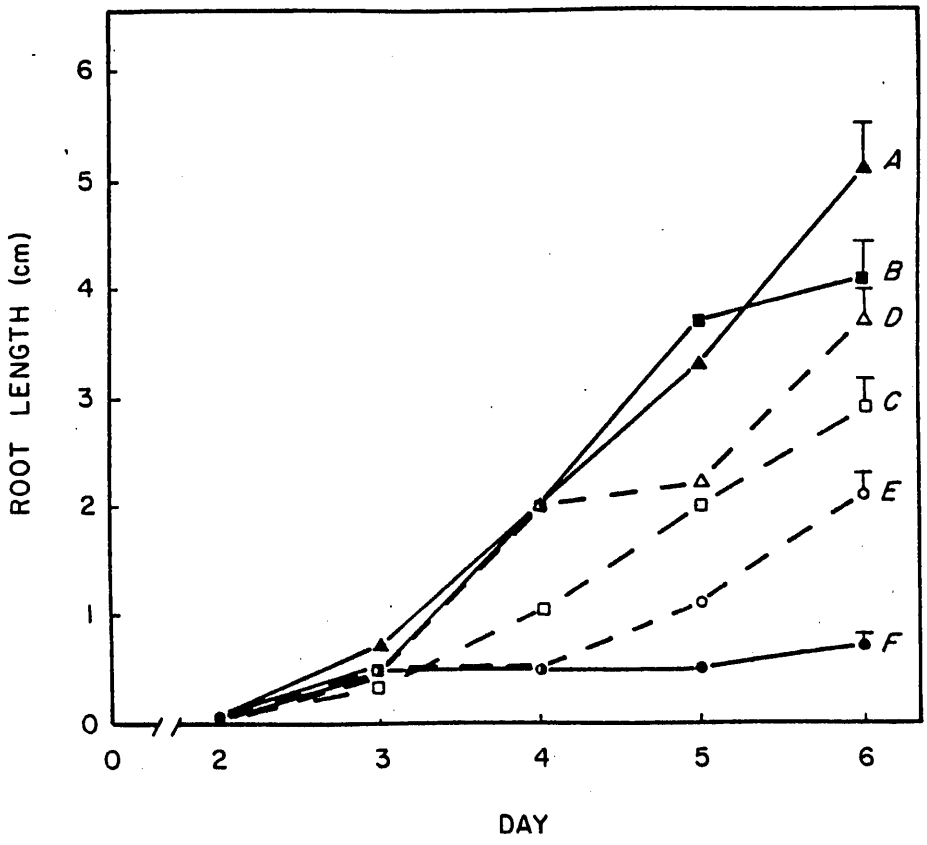

Figure III.3 The effect of 0.3 μℓ ℓ^{-1} SO_2 on root elongation of tomato cultivar VF 134 seedling fumigated four hours daily for 0, 2, 4, or 6 days at 25°C. Control (A). SO_2 fumigation on day 1 and 2 (B); day 3 and 4 (C); day 1, 2, 3, 4 (D); day 1, 2, 3, 4, 5, 6 (E).

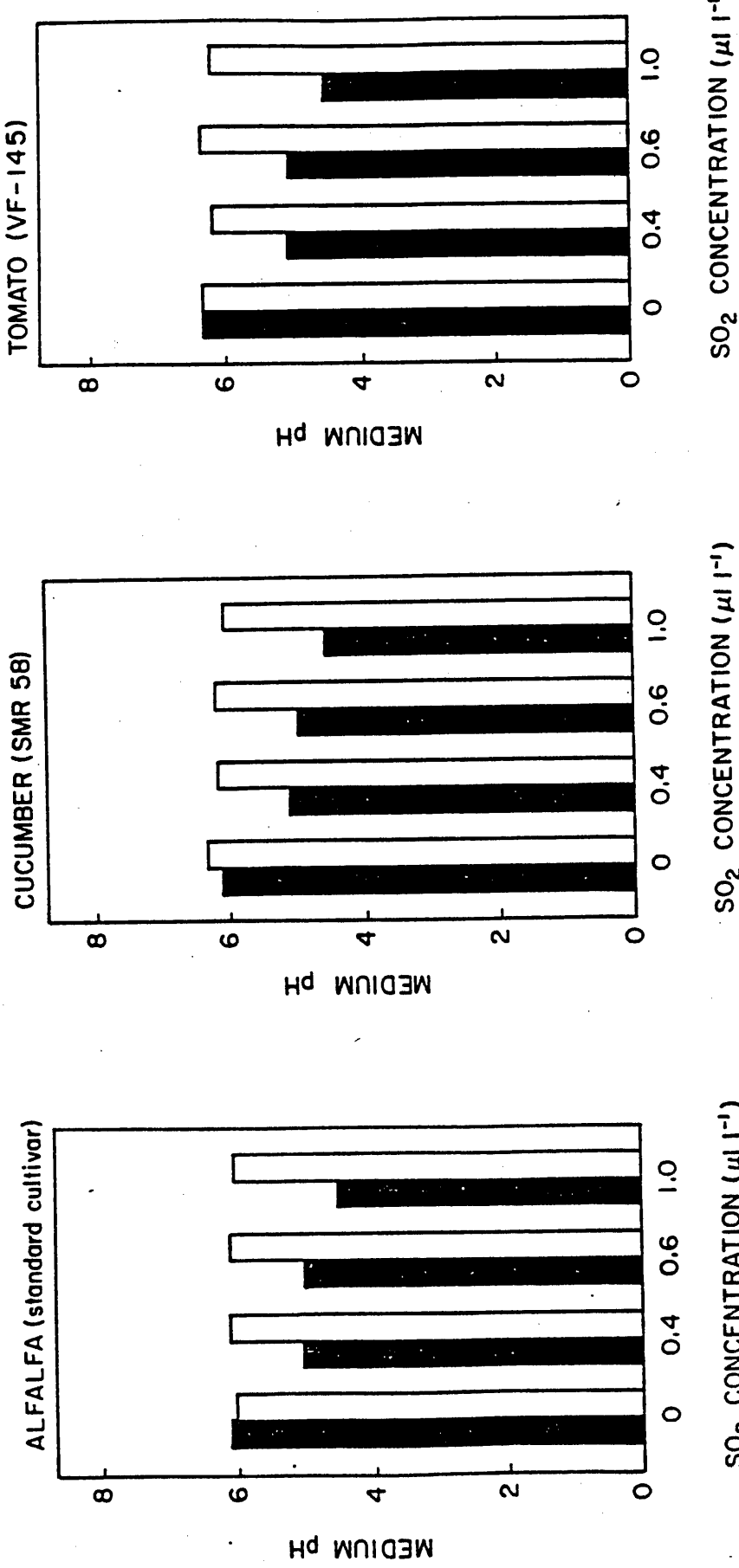

Figure III.4 The effect of two hours of fumigation with four levels of SO_2 on pH of two media, buffered (white) and unbuffered (black), using three species of pollen, alfalfa, cucumber, and tomato.

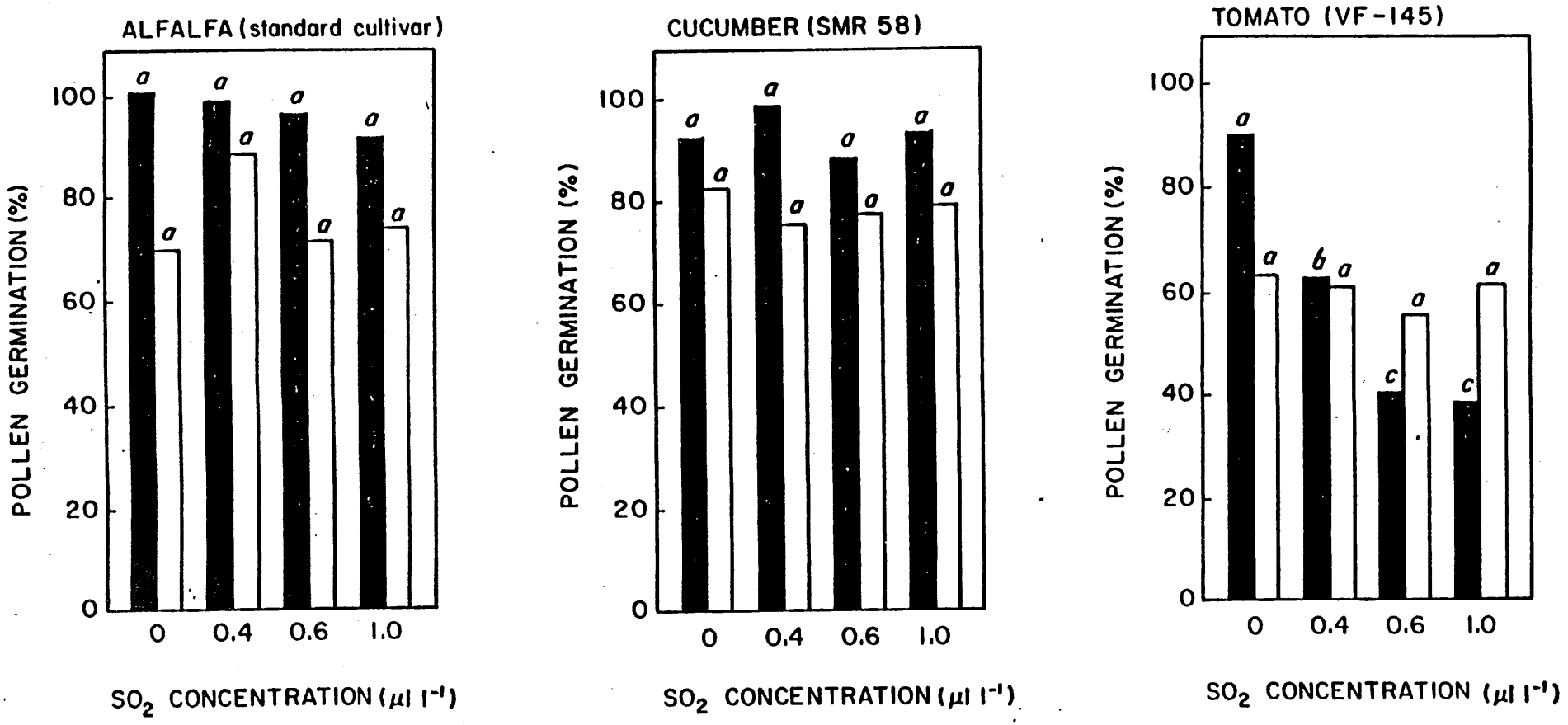

Figure III.5 The effect of two hours of fumigation with four levels of SO_2 on percent pollen germination of alfalfa, cucumber, and tomato grown on two media, buffered (white) and unbuffered (black). Within each type of medium, bars sharing a common letter are not significantly different at the 5% level.

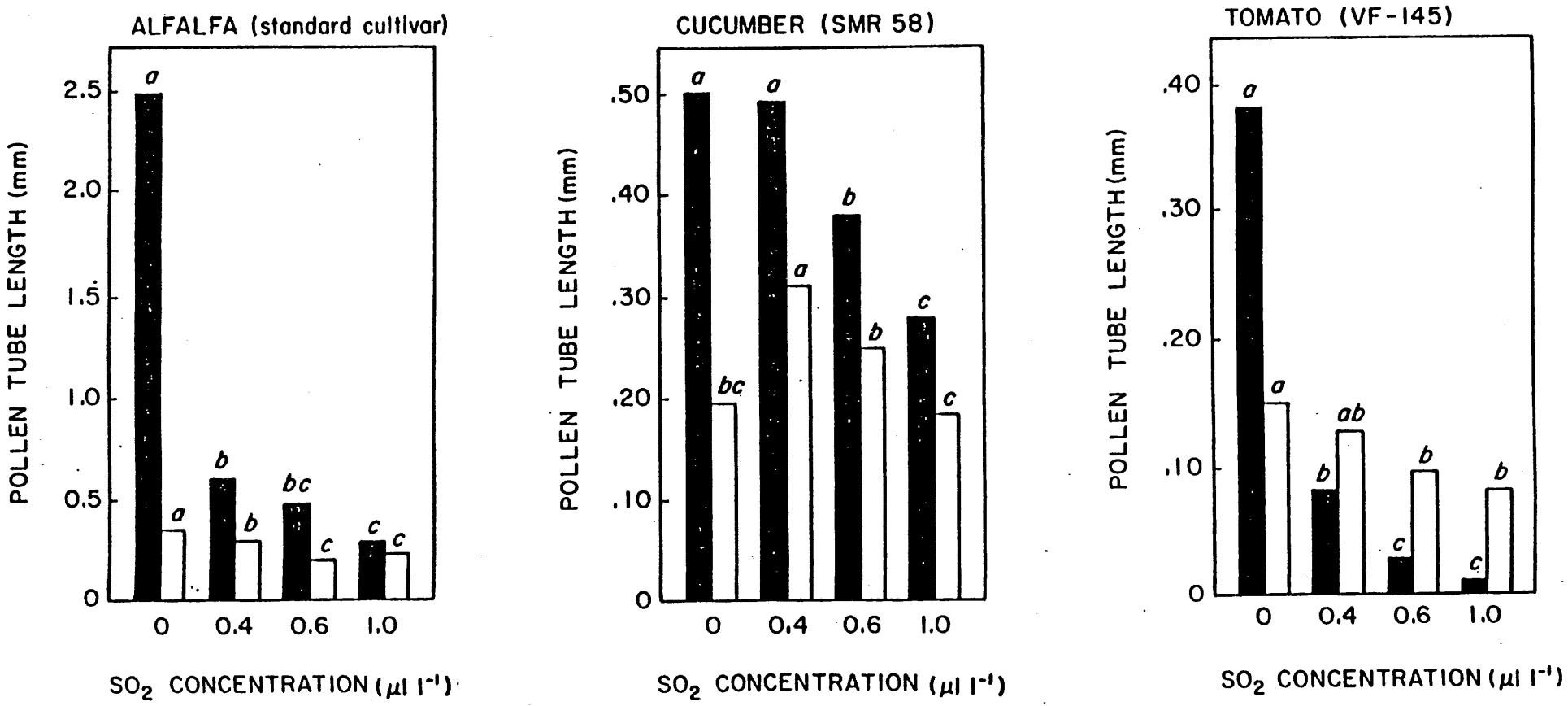

Figure III.6 The effect of two hours of fumigation with four levels of SO_2 on pollen tube length of alfalfa, cucumber, and tomato grown on two media, buffered (white) and unbuffered (black). Within each type of medium, bars sharing a common letter are not significantly different at the 5% level.

CHAPTER IV. Comparative Development: Corn

1. Introduction

Plant response to gaseous air pollution may vary with pollutant type (Miyake et al., 1984), plant species (Tingey et al., 1973), cultivars within a species (Cameron and Taylor, 1973; Butler and Tibbitts, 1979), age of tissue (Ting and Dugger, 1968; Craker and Starbuck, 1973), and environmental factors (Taylor, 1974). This study involved three corn cultivars that have previously been described as differentially sensitive to ozone (Cameron and Taylor, 1973; Thompson et al., 1976). The sensitivity of these cultivars to SO_2 under controlled conditions was investigated at three developmental stages: germination, young seedlings, and fully-developed plant. Certain physiological parameters were selected as possible indicators or markers that could be correlated with growth and injury responses.

The role of stomata in the resistance of plants to air pollution stress has been well documented (Mansfield and Freer-Smith, 1984). Stomatal aperture controls the flux of gaseous substances into the leaf where accumulation and damage to membranes and metabolism may occur. Interference with the stomatal mechanism has been reported for SO_2 (Mansfield and Majernik, 1970) and O_3 (Olszyk and Tibbitts, 1981). Conflicting reports on whether stomata respond to air pollutants by opening or closure have been reported (Black, 1982). This variable effect may be due to the pollutant level, environmental factors such as relative humidity (McLaughlin and Taylor, 1981), or plant species. Whatever the case may be, alteration of the stomatal mechanism will have an effect on the growth and metabolism of the plant. If the stomatal aperture is increased by the gaseous pollutant, the flux of the gas into the leaf will increase leaving the plant vulnerable to internal metabolic disruption and increased transpiration. If the stomatal aperture is decreased, the supply of CO_2 to the leaf - the site of photosynthesis - may become limiting, resulting in growth inhibition. The effects of gaseous pollutants on the stomatal mechanism may be reflected by changes in the water relations of the plant. For these reasons we investigated the stomatal responses of the three corn cultivars to low levels of SO_2. Correlation of

stomatal responses with physiological parameters related to water relations of the plants were also investigated.

Further clarification of the role of stomata in air pollutant response was obtained by fumigating very young seedlings that had not yet developed stomatal complexes. A correlation of the plant responses was sought between the two growth stages.

2. Materials and Methods

Seed Germination and Seedling Growth

Seeds of three corn cultivars, two of which have been described as ozone resistant, NC+59 (NC) and Bonanza (B), and the other as ozone sensitive, NK 51036 (NK) (Cameron and Taylor, 1973), were germinated and grown on filter paper saturated with distilled water or a 0.005 M solution of MES (2 N-morpholiro ethanesulfonic acid). The use of distilled water resulted in acidification of the growth medium during SO_2 fumigation but there was no change in pH when MES-buffer was used (Table IV.1).

Seeds were imbibed in distilled water for four hours prior to placement on the filter paper. The seeds were sandwiched between two layers of filter paper, supported by a glass frame, with the lower edge of the paper standing in a container of the appropriate solution.

Fumigation commenced upon placement of the germination paper into 16 ℓ glass fumigation chambers (both the gas delivery system and the chambers are described in detail in Chapter IB). The chambers were placed in a constant temperature room at 25°C. Sulfur dioxide and ozone treatments consisted of SO_2 (0.2 to 2.0 $\mu\ell\ \ell^{-1}$) or ozone (0.14 $\mu\ell\ \ell^{-1}$) alone or mixed for 4 hours daily until the coleoptile emerged.

A prepared pad of each cultivar was placed in 12 fumigation chambers. When SO_2 was applied, 4 levels of SO_2 were replicated three times. When fumigation with SO_2 and O_3 was tested, three levels of SO_2 and two levels of O_3 (0 and 0.14 $\mu\ell\ \ell^{-1}$) were replicated two times. A minimum of three separate and sometimes five separate repeated fumigation runs were conducted.

Additional Experiments

These were performed on two-week old corn seedlings of the three corn cultivars described above. Seeds were imbibed for 24 hours in aerated deionized water in the dark at room temperature. They were then sown on a layer of washed cheesecloth supported by a stainless steel screen suspended approximately 1 cm above the surface of an aerated 0.2 mol m^{-3} $CaSO_4$ solution and allowed to grow for two days in the dark. On the fourth day, the seedlings were exposed to laboratory light conditions. After 24 hours in the light, the seedlings were moved to a greenhouse or, in the case of the experiments on the time course of stomatal conductance, to a growth chamber. At this time, the seedlings were transferred to 3.8 ℓ plastic containers filled with aerated nutrient solution. Nine plants were placed in each container and supported by plastic grids. The nutrient solution was a modified Hoagland solution (Epstein, 1972) at 0.1 strength with Fe-EDTA at 0.2 strength. The seedlings were allowed to grow for one week. The nutrient solution was changed on the third and seventh day to fresh 0.5 strength Hoagland solution with full strength Fe-EDTA. When the seedlings were eleven days old, they were transferred to exposure chambers (described below) housed in a controlled environment growth chamber. Three containers per cultivar were placed in each of the two exposure chambers. The seedlings were allowed to acclimate to the environmental conditions of the exposure chamber for three days prior to fumigation.

Fumigation Conditions: The fumigation chambers were 0.61 m x 0.61 m x 0.813 m wood framed boxes covered with transparent teflon film (Fig. IV.1). The chambers were housed in a larger controlled environment growth chamber. The control of light, temperature and humidity (RH) conditions in the fumigation chambers was accomplished through the control of these conditions in the larger growth chamber. The environmental conditions in the fumigation chambers were as follows, photoperiod 16 hr; quantum flux density, measured with Lambda Quantumeter, 250 ± 10 μ Einsteins $m^{-2}s^{-1}$ (400-700 nm) at the middle of the plant canopy; 25.8 ± 1°C day/19.5 ± 0.5°C night temperature; and 65 ± 5% day/85 ± 5% night RH. Air temperature was measured in the middle of the plant canopy continuously with a Cu-Constantan

thermocouple in each chamber connected to a data logger. RH was monitored with a Weathermeasure, HM-111, solid state humidity sensor.

The air entering the fumigation chambers was charcoal filtered and drawn into the chambers with a blower motor mounted on the exterior of the growth chamber and ducted to the exposure chambers (Fig. IV.1). The rate of air exchange was controlled by Butterfly valves positioned below the charcoal filters and was set at 240 ℓ/min. Sulfur dioxide was supplied from a cylinder containing 1,500 $\mu\ell\ \ell^{-1}$ SO_2 and provided a concentration range from 0.2 to 1.5 $\mu\ell\ \ell^{-1}$ in the fumigation chamber. The SO_2 concentration in the exposure chamber was sampled in the middle of the plant canopy with a Thermo Electron pulsed fluorescent SO_2 analyzer. SO_2 was fed into a mixing plenum prior to their introduction into the fumigation chamber.

The plants were exposed to SO_2 at two concentration levels, 0.5 $\mu\ell\ \ell^{-1}$ and 1.5 $\mu\ell\ \ell^{-1}$ for six hours. Subsequent experiments examined the effects of long-term, low-level exposures to SO_2. Plants were exposed to 0.48 $\mu\ell\ \ell^{-1}$ SO_2 for six hours on four consecutive days. SO_2 fumigations were initiated routinely at two hours into the photoperiod.

Leaf Area Measurements: Leaf areas were determined with a LiCor Leaf Area Meter, Model LI 3000. Leaf traces showing necrotic areas were made of the third and fourth leaves of each plant. The leaf traces were then cut out and their areas measured. Then, the necrotic areas were cut away and the leaf traces were remeasured to assess the amount of damage.

Stomatal Density: Stomatal densities of the upper and lower surfaces of the third leaf were determined from microscopic examinations of leaf impressions. The leaf impressions were made by spraying the leaf surfaces with three layers of clear plastic acrylic paint followed by three layers of clear lacquer. The resulting layers of film over the leaves were then peeled off with fine forceps and mounted on microscope slides for the counting of stomata under a stereoscopic microscope.

Stomatal Conductance Measurements: Diffusive conductance of stomates was measured in the middle of the third leaf of each plant on the abaxial side with a Lambda autoporometer and LI-20s horizontal

sensor. Calibration of the sensor was performed according to T. C. Hsiao (personal communication). The sensor was stored in a desiccator over silica gel between experiments. Prior to measurements, the sensor was placed into the exposure chambers and allowed to come to thermal equilibrium. Measurements were made through glove ports in the sides of the exposure chambers. Only one measurement was made per plant.

Leaf Water Potential (Ψ leaf measurements): The Ψ leaf of corn plants was measured with a pressure bomb (Scholander et al., 1965). The leaves were excised at the base with a razor blade and immediately placed in the bomb 24 hr following fumigation treatments.

Osmotic Potential (Ψ_π) Measurements: Shoot and root osmotic potentials were measured with a Wescor vapor pressure osmometer (Model 5100 A). Shoot and roots were harvested 24 hours after fumigation. The roots were rinsed with deionized water, gently blotted dry and immediately frozen along with excised shoots. The shoot and root samples were later wrapped individually with washed and dried cheesecloth and then pressed with a Carver Laboratory Press at 3,000 lb in^{-2} for a period of 5 min. The sap samples were collected with Pasteur pipettes and stored frozen in 4 ml glass vials until analyzed.

3. Results

Germination

No reduction in germination resulted from a daily 4-hour fumigation with the SO_2 concentrations used in this study.

Root Growth

Root elongation of two corn cultivars Bonanza and NC+59 was significantly reduced when exposed to 0.5 $\mu\ell\ \ell^{-1}$ SO_2, when either distilled H_2O or MES-buffer was used (Fig. IV.2). Cultivar NK 51036 was less sensitive to 0.5 $\mu\ell\ \ell^{-1}$ SO_2. Root elongation of all cultivars was markedly retarded above 0.6 $\mu\ell\ \ell^{-1}$ SO_2. Greater retardation was found with MES-buffer as the root medium than with distilled water.

The percent reduction (relative to controls) of root growth of plants fumigated with SO_2 at 0.4 and 0.8 $\mu\ell\ \ell^{-1}$ for four hours daily

is shown in Table IV.2. Differences in cultivar response to the two germination media are apparent. NK 51036 is similarly affected by SO_2 fumigation on both media. Cultivar Bonanza is more affected by SO_2 when grown on MES-buffer at 0.8 $\mu\ell\ \ell^{-1}$ SO_2, but not at 0.4 $\mu\ell\ \ell^{-1}$ SO_2. In contrast, root extension of NC+59 is less sensitive to 0.4 $\mu\ell\ \ell^{-1}$ SO_2 when grown on MES-buffer, but more sensitive on MES-buffer at 0.8 $\mu\ell\ \ell^{-1}$ SO_2.

Root elongation of cultivars NK 51036 and NC+59 was significantly more reduced when fumigated with a mixture of 0.14 $\mu\ell\ \ell^{-1}$ O_3 and 1.0 $\mu\ell\ \ell^{-1}$ SO_2 compared to fumigation with 1.0 $\mu\ell\ \ell^{-1}$ SO_2 without O_3 (Fig. IV.3). No similar interaction occurred at 0.5 $\mu\ell\ \ell^{-1}$ SO_2 and 0.14 $\mu\ell\ \ell^{-1}$ O_3. Root elongation of cv. Bonanza seedlings was adversely affected by the presence of SO_2. The addition of O_3 did not further affect root elongation. Using the factorial analysis and by partitioning the treatment sum of squares into main effects and interactions (Little, 1981), 70% of the response variability was attributable to SO_2, 10% to SO_2 x O_3, and 5% to cultivar.

Visible Injury

Injury to the various cultivars, induced by SO_2, was assessed by leaf traces of the necrotic regions of the third and fourth leaves 24 hours after fumigation. Treatments of 1.5 $\mu\ell\ \ell^{-1}$ SO_2 for six hours caused severe visible injury to the leaves of both the NC+59 and Bonanza cultivars, but very little to the same leaves of the NK 51036 cultivar (Table IV.3). None of the three cultivars examined displayed visible injury when exposed to 0.5 $\mu\ell\ \ell^{-1}$ SO_2 for six hours (data not shown).

Stomatal Density

The stomatal densities on the upper (adaxial) and lower (abaxial) sides of corn leaves were measured to determine the relation between stomatal density and cultivar sensitivity to SO_2 (Table IV.4). The stomatal density, on the upper leaf side of the NK 51036 plants was the highest of the three cultivars (43.8 stomates mm^{-2}) while the densities of the Bonanza and NC+59 cultivars were lowest and approximately equal (36.4 and 35.9 stomates mm^{-2}, respectively). On the lower leaf sides, the Bonanza cultivar plants had the highest stomatal density and the NK 51036 plants the lowest stomatal density

(60.6 and 54.0 stomates mm^{-2}). The total average (upper and lower) stomatal densities was the highest in the NK 51036 and Bonanza cultivars while the lowest average density was found in the NC+59 cultivar plants.

Effects of 1.5 $\mu\ell\ \ell^{-1}$ SO_2 (6 hours).

Stomatal Conductance. Differences among the cultivars in stomatal conductance were found after six hours of fumigation with 1.5 $\mu\ell\ \ell^{-1}$ SO_2. Significant stomatal closures were observed in both the NC+59 and NK 51036 cultivars, but not in the Bonanza cultivar (Table IV.5). These results were found to be extremely reproducible in replicate experiments.

Prior to fumigation, stomatal conductance was highest in the NC+59 and lowest in the NK 51036 cultivar plants (Figs. IV.4a,b,c). In the NC+59 plants (Fig. IV.4a), the stomatal conductance of both the control and SO_2 treated plants decreased with time. The conductance of the SO_2 fumigated plants, however, dropped significantly more than the control plants by the sixth hour of exposure. The stomatal conductance of both the SO_2 treated and control plants of the Bonanza cultivar also decreased with time but did not become significantly different from one another during the exposure period (Fig. IV.4b). In the SO_2 treated NK 51036 plants (Fig. IV.4c), stomatal conductance was reduced compared to the control plants after only two hours of SO_2 exposure. This suppressed level of conductance was maintained throughout the remainder of the fumigation period.

To examine possible long-term effects of SO_2 on stomatal conductance, conductance was examined at various times after the exposure of the three cultivars to 1.5 $\mu\ell\ \ell^{-1}$ SO_2 for six hours to determine if any permanent damage had been done to the stomates by SO_2. Conductance was measured immediately after SO_2 exposure, one hour after the termination of the photoperiod and two hours after the photoperiod resumed the morning after the exposure (Fig. IV.5). In all three cultivars, the conductance of SO_2 treated plants appeared to return to their normal functional capacity.

Water Potential Measurements (leaf Ψ). In an attempt to examine the secondary effects of altered stomatal conductance the leaf Ψ of SO_2 exposed plants was measured. Differences in leaf Ψ were only

observed in the Bonanza cultivar after exposure to 1.5 $\mu\ell\ \ell^{-1}$ SO_2 for six hours, with SO_2 causing an increase in Ψ. No significant differences in leaf Ψ were observed for the NK 51036 and NC+59 cultivars (Table IV.6).

Osmotic Potential (Ψ_π) Studies. Osmotic potentials of the shoots and roots of the three corn cultivars were examined after the plants were exposed to 1.5 ppm SO_2 for 6 hours. Only the roots of the NK 51036 cultivar showed a significant osmotic potential difference (-4.8 vs. -5.4 bars for the SO_2 and control roots, respectively).

Low-Level SO_2 Effects (0.5 $\mu\ell\ \ell^{-1}$ SO_2, 6 hours). Stomatal closure occurs after low-level SO_2 fumigation in both the NC+59 and NK 51036 cultivars (Table IV.7). There was no significant effect, however, on stomatal conductance of the cultivar Bonanza.

Effects of Long-Term, Low-Level SO_2 Fumigation on Growth and Water Relations. In these experiments, two week old NK 51036, Bonanza, and NC+59 plants were exposed to 0.48 $\mu\ell\ \ell^{-1}$ SO_2 for six hours on four successive days then analyzed for differences in fresh weight, shoot and root osmotic potential, and leaf water potential (Table IV.8a,b,c). No signs of visible injury were apparent after these long-term SO_2 exposures.

Fresh Weight. Significant differences in fresh weights were observed in SO_2 treated and control plants of the NC+59 and Bonanza cultivars but not in the NK 51036 cultivar (Table IV.8a). Lower than control fresh weights were measured for the SO_2 treated shoots of the Bonanza and NC+59 plants and the roots of the NC+59 plants. No significant differences in fresh weights were recorded for the shoots or roots of the NK 51036 plants and the roots of the Bonanza plants.

Shoot and Root Osmotic Potentials (Ψ_π). In the SO_2 treated plants, only the Ψ_π of the roots of the NK 51036 cultivar and Ψ_π of the shoots of the NC+59 cultivar were significantly different from the control plants (Table IV.8b). In the SO_2 treated NK 51036 plants, the Ψ_π of the roots were lower (more negative) than the control plants; and the Ψ_π of the shoots of SO_2 treated NC+59 plants was higher than control plant values.

Leaf Water Potential (leaf Ψ). Significant differences in leaf Ψ values were found between the control and SO_2 treated corn cultivars

of Bonanza and NC+59 but not in the NK 51036 cultivar plants (Table IV.8c). The leaf Ψ of the SO_2 treated Bonanza and NC+59 plants were both higher (49 and 61%, respectively) than control values.

4. Discussion

Within two days of seed placement in the germination facility, radicle growth commenced. There was no effect by any level of SO_2 on radicle protrusion. Whether this was due to the initial imbibition in distilled water, which may have allowed the seed to reach a metabolic stage less sensitive to SO_2, or to the resistance of the seed coat to SO_2 or HSO_3^- uptake is unclear and requires further study.

Subsequent root growth was clearly affected by SO_2 and O_3, singly or in combination. Tingey et al. (1971) attributed the reduced root growth of fumigated radish plants to a decline in carbohydrate synthesis. In this study root growth was apparently affected by the formation of HSO_3^- as well as by the depressed pH of the germination media. The absence of Ca^{2+} in the germination media probably enhanced the deleterious effect of increased H^+ concentration at low pH in the distilled water media (Foy, 1974). Though reduced pH was a component contributing to the root growth retardation, it is noteworthy that over the SO_2 levels employed in this investigation root growth was inhibited more in plants grown on MES-buffer than in those grown on distilled water (Fig. IV.2). At the pH level maintained by the MES-buffer (6.4), the formation of HSO_3^- is favored (Spedding et al., 1980). Absorption of HSO_3^- by the rapidly developing roots may lead to toxic concentrations in the plant tissue. The ability of the seedling to detoxify HSO_3^- may be an important component of resistance at this growth stage. Table IV.2 shows the effects of the two germination media at two fumigation levels for all three cultivars. The contrasting sensitivity of the three cultivars to the SO_2 level and the pH of the germination media may be important in determining sensitive physiological sites to SO_2 action. It may be hypothesized that two different mechanisms may be involved in the sensitivities of cvs. Bonanza and NC+59 to the two media and the two concentrations of SO_2, respectively (Table IV.2).

Fumigation with 0.14 μl ℓ^{-1} ozone for four hours daily, in the absence of SO_2, did not affect the root growth of the three corn cultivars at seedling growth stage (Fig. IV.3). This agrees with the previously reported ozone resistance of cultivars NK 51036 and Bonanza at a more mature growth stage (Cameron and Taylor, 1973; Thomson et al., 1976), but not with the reported sensitivity of NC+59 (Cameron and Taylor, 1973). It has been suggested that young leaves are more resistant to SO_2 than older leaves even though the younger leaves absorb more SO_2 (Bressan et al. 1978, 1979; Tanaka and Sugahara, 1980). Tanaka and Sugahara (1980) reported higher levels of superoxide dismutase (a free-radical scavenger) activity in young leaves than in older leaves. Free radicals have been implicated as agents in ozone damage to plant metabolism (Tingey and Taylor, 1982). In bean plants exposed to ozone, superoxide dismutase was reported to act as a protective agent (Lee and Bennett, 1982). The young root tissue in our investigations may have had higher levels of superoxide dismutase, or the germination system employed may have inhibited the uptake of ozone or its dissociation products by the plant roots. Further study is required to fully explain the discrepancy in O_3 resistance of cultivar NC+59 at different growth stages.

The addition of 0.14 μl ℓ^{-1} O_3 significantly reduced the root elongation of cultivars NK 51036 and NC+59 from that of plants fumigated with SO_2 alone (Fig. IV.3). This effect was not apparent with fumigation of the aforementioned O_3 concentration and 0.5 μl ℓ^{-1} SO_2. Cultivar Bonanza was unaffected by the addition of ozone to SO_2 (Fig. IV.3). The effects of air pollutant mixtures have recently been reviewed by Ormrod (1982). Plant responses to various air pollutant mixtures have been described as synergistic, additive, or antagonistic (Ormrod, 1982). These discrepancies may be explained by differences in pollutant dose, plant species, developmental stage, and experimental conditions. The interactions of the gaseous species will govern plant response. For example, it has been suggested (Rich, 1964) that the initial site of attack by ozone or its derivatives is the cell membrane and, therefore, transport processes may be disrupted (Heath and Frederick, 1979; Perchorowicz and Ting, 1974). Excessive amounts of HSO_3^- or SO_3^{2-}, from SO_2 dissociation, may enter the cell

and interfere with metabolism. Another possible site of interaction is the sub-stomatal cavity where stomatal aperture may be modified unexpectedly, drastically affecting gas exchange and water relation characteristics of the plant. The latter possibility has been eliminated from the seedling investigation since the endpoint of these experiments preceded stomatal function. The third possibility is an interaction of the fumigant gases prior to deposition on or uptake by the plant. This appears unlikely because of the differential cultivar responses of seedlings grown under identical conditions. Though the first possibility seems most likely to apply to this particular investigation, it is beyond the scope of this study to ascertain the mechanism conferring differential resistance to ozone and SO_2 fumigation.

Concurrent experiments were carried out using the same three corn cultivars at a photosynthetically active growth stage. A single six hour fumigation in the light with 1.5 $\mu\ell\ \ell^{-1}$ SO_2 induced visible injury to a greater extent in cultivars Bonanza and NC+59 than in NK 51036 (Table IV.3). The primary site of SO_2 uptake is the stomata (Majernik and Mansfield, 1970). Sulfur dioxide has been shown to affect stomatal function, resulting in enhancement (Mansfield and Majernik, 1970; Unsworth, Biscoe and Pinckney, 1972) or depression (Kondo and Sugahara, 1978) of conductance. The range of stomatal responses reported has been attributed to plant species, concentration and duration of SO_2 exposure, and environmental conditions (Black, 1982). Stomatal density might be expected to give a rough correlation with SO_2 uptake, and possibly visible injury. This did not appear to be a good indicator for the cultivars employed in this investigation. In fact, stomatal density was higher on the adaxial surface of the NK 51036 leaves than on the other cultivars' adaxial leaf surfaces (Table IV.4). Abaxial stomatal density of cultivar NK 51036 was significantly lower than the other two cultivars, but in terms of visible injury average stomatal density (Table IV.4, column 3) did not appear to be related to cultivar response.

A more accurate parameter for assessing SO_2 uptake is stomatal conductance. Significant reductions in stomatal conductance following fumigation that induced visible injury (1.5 $\mu\ell\ \ell^{-1}$, 6 hr) were

observed in the cultivars NK 51036 and NC+59 (Table IV.5). Even though NC+59 experienced a significant reduction, the post-fumigation conductance was still significantly higher than in cultivar NK 51036 (Table IV.5). The stomatal conductance of cultivar Bonanza was also significantly higher than that of cultivar NK 51036.

Further clarification of stomatal responses to SO_2 fumigation was accomplished by following conductance through a fumigation period (Fig. IV.4a,b,c) and examining the recovery time of stomatal function (Fig. IV.5a,b,c). Stomatal conductance of cultivar NK 51036 fumigated plants was significantly reduced after 1.5 hours of exposure and stayed that way for the remainder of the fumigation period (Fig. IV.4c). Cultivar NC+59 fumigated plants did not exhibit significantly reduced stomatal conductance until the conclusion of the fumigation period (Fig. IV.4a). The combination of rapid stomatal closure and low intrinsic conductance appears likely to be a significant factor in the relative resistance of cultivar NK 51036 to visible injury at this SO_2 level. Stomatal conductance of all three cultivars returned to control levels by the morning following fumigation (Fig. IV.5a,b,c).

Sulfur dioxide fumigation at a level that did not cause visible damage (0.5 $\mu\ell\ \ell^{-1}$, 6 hr) had an effect on stomatal conductance, as well. Stomatal closure was induced in the cultivars NK 53106 and NC+59 (Table IV.7) as it was at 1.5 $\mu\ell\ \ell^{-1}$ SO_2. Unlike the response at the higher SO_2 concentration, conductance values for cultivar NC+59 were reduced to the level of NK 51036, although the initial conductance was much higher (Table IV.7). Cultivar Bonanza was again unaffected.

Fumigation of plants at 0.48 $\mu\ell\ \ell^{-1}$ SO_2 for six hours on four successive days was employed to investigate growth and water relation parameters of the corn cultivars as affected by long-term low-level SO_2 fumigation. The protection from SO_2-toxicity afforded a plant by reduced stomatal aperture may have undesirable consequences on growth and yield. This phenomenon did not appear to be in effect as far as cultivar NK 51036 was concerned. After four days of SO_2 exposure neither root nor shoot fresh weights were diminished in this cultivar (Table IV.8a). This may have been due to corn's ability to fix carbon fairly efficiently at low CO_2 concentrations because of its C-4

photosynthetic pathway. Shoot fresh weights of cultivars Bonanza and NC+59 were significantly reduced from control levels by SO_2 fumigation. Root fresh weight in cultivar NC+59 was also significantly reduced (Table IV.8a).

Water relation parameters of plants that had altered stomatal behavior would be expected to be affected. Single exposure to SO_2 did not bear out this assumption. The leaf water potential of plants exposed to 1.5 (Table IV.6) or 0.5 (data not shown) $\mu\ell\ \ell^{-1}$ SO_2 for six hours was unaffected except in cultivar Bonanza at 1.5 $\mu\ell\ \ell^{-1}$ SO_2 where it became significantly less negative even though Bonanza's stomatal conductance was unaffected by either treatment. Multiple fumigations with 0.5 $\mu\ell\ \ell^{-1}$ SO_2 resulted in an increase in leaf water potential of cultivars Bonanza and NC+59 (Table IV.8c) which correlates with the reduction in shoot fresh weight observed (Table IV.8a).

The reduction in osmotic potential (Ψ_π) of the SO_2 treated NK plants was surprising, but perhaps informative. Although there was no correlation between the reduced Ψ_π and injury or reduced growth, the reduction in Ψ_π of the roots (Table IV.8b) may instead be related to the reduced stomatal conductance. In the NC cultivar there was a similar, though not highly significant, reduction in the Ψ_π of the roots of the SO_2 treated plants. The lack of measurable Ψ_π difference in the shoots of the three cultivars may be a result of isolated changes that only occur in the most sensitive organs, the third and fourth leaves. By using the entire shoot to obtain Ψ_π measurement, we may have not been able to discern localized changes in Ψ_π.

To clarify resistance mechanisms in corn, it may be significant to note the similar responses cultivars made to SO_2 exposure at two differing stages of development: germinating seedling and juvenile. Stomatal responses did not seem to account for the differential sensitivity of the three cultivars to acute or chronic exposures to SO_2. Further emphasis is placed on this point by the similar response of the three cultivars at the young seedling stage where stomata are non-functional. Detoxification or differential uptake at the cellular level may be of greater importance in accounting for differences in cultivar sensitivity to SO_2 exposure. Further investigation is

required to characterize actual SO_2 uptake rates, and detoxification parameters of physiologically active species of SO_2.

5. Summary

Three corn cultivars that had been previously described as differentially sensitive to ozone were examined to ascertain their response to various levels of SO_2 and O_3, alone or in combination. Visible injury, growth, and various water relation parameters were investigated at different growth stages. Differential cultivar sensitivity to SO_2, in terms of visible injury and growth, differed from that to ozone as described in the literature. Sensitivity of the corn cultivars to SO_2, however, was similar at two distinct growth stages. Similarity in sensitivity at these distinct stages of growth, one without functional stomata (young seedling and the other with functional stomata), may be significant in identifying resistance mechanisms. Differential SO_2 uptake rates at the cellular level and/or SO_2 detoxification may be more important factors in determining a plant's sensitivity to SO_2 than stomatal control of its uptake.

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Table IV.1 Comparison of pH of media in root zone of corn seedlings germinated in distilled water and in MES-buffer medium (pH 6.4) after four hours daily fumigation for five days with SO_2 at 25°C.

SO_2 ($\mu\ell\ \ell^{-1}$)	pH	
	H_2O	MES
0	5.9	6.4
0.4 ± 0.05	4.5	6.4
0.8 ± 0.2	4.1	6.4

Table IV.2 Comparative root growth response to SO_2 fumigation of corn seed germinated on distilled water and on MES-buffer medium (pH 6.4).

Cultivar	SO_2 ($\mu\ell\ \ell^{-1}$)	Percent root growth* (control = 100%)	
		H_2O	MES
NK 51036	0.4 ± 0.05	91a	91a
	0.8 ± 0.2	62a	65a
Bonanza	0.4 ± 0.05	79a	77a
	0.8 ± 0.2	75a	51b
NC+59	0.4 ± 0.05	71a	90b
	0.8 ± 0.2	68a	46b

*Values followed by a common letter are not significantly different at the 5% level.

Statistical analysis was based on Duncan's multiple range test after significance by analysis of variance.

Table IV.3 Comparison of injury response on the third and fourth leaves of three corn cultivars (1.5 μℓ $ℓ^{-1}$ SO_2, 6 hr).

Cultivar	% Necrosis*		
	Leaf No. 3	Leaf No. 4	Total Ave.
NK 51036	0.6b	0.4b	0.5b
Bonanza	3.9a	4.5a	4.2a
NC+59	3.6a	3.7a	3.7a

*Values followed by a common letter are not significantly different at the 5% level. Each value is the mean of six leaves.

Table IV.4 Comparison of stomatal densities on the adiaxal and abaxial leaf surfaces of the third leaf of three corn cultivars.

Cultivar	Stomatal Density (No. mm^{-2})*		
	Adaxial	Abaxial	$\frac{\text{Adaxial + Abaxial}}{2}$
NK 51036	43.8a	54.0c	48.8a
Bonanza	36.4b	60.6a	48.5a
NC+59	35.9b	57.0b	46.4b

*Values followed by a common letter are not significantly different at the 5% level. Each value is the mean of ten microscope fields per leaf for five leaves.

Means compared within columns and rows.

Table IV.5 Effects of SO_2 on stomatal conductance of three corn cultivars following fumigation (1.5 μℓ $ℓ^{-1}$, 6 hr).

Cultivar	Stomatal Conductance (cm sec^{-1})*			
	Control	SO_2 treated	Net difference	% difference
NK 51036	0.23bx	0.11by	-0.12	-47.8
Bonanza	0.25bx	0.19ax	-0.06	-24.0
NC+59	0.36ax	0.20ay	-0.16	-41.9

*Cultivar differences: values followed by a common letter (a-b) are not significantly different at the 5% level.

Treatment effects within cultivars: values followed by a common letter (x-y) are not significantly different at the 5% level.

Each value is the mean of eight measurements on eight plants.

Means compared within columns and rows.

Table IV.6 Effects of SO_2 on leaf water potential (leaf Ψ) of three corn cultivars.

Cultivars	1.5 $\mu\ell\ \ell^{-1}$ SO_2, 6 hr		Paired t-test: % level of significance
	Ψ (bars)		
	Control	SO_2 Treated	
NK 51036	-1.250 ± 0.204	-1.250 ± 0.440	36.32
Bonanza	-2.188 ± 0.315	-1.625 ± 0.250	3.80*
NC+59	-2.250 ± 0.289	-2.063 ± 0.427	49.75

*Significant at <5%. Statistically analyzed with paired T-test.

Each value if the mean of four replicate plant samples.

Table IV.7 Effects of SO_2 on stomatal conductance of three corn cultivars (0.5 $\mu\ell\ \ell^{-1}$, 6 hr).

Cultivar	Stomatal Conductance (cm sec^{-1})*			
	Control	SO_2 Treated	Net Difference	% Difference
NK 51036	0.22bx	0.14by	-0.08	-36
Bonanza	0.21bx	0.25ax	0.04	+19
NC+59	0.35ax	0.15by	-0.20	-57

*Cultivar differences: values followed by a common letter (a-b) are not significantly different at the 5% level.

Treatment effects within cultivars: values followed by a common letter (x-y) are not significantly different at the 5% level.

Each value is the mean of eight measurements made on eight plants.

Means compared within columns. Percent difference significant at 5% level.

Table IV.8 Effects of multiple low-level SO_2 fumigations (0.48 $\mu\ell\ \ell^{-1}$, 6 hr, 4x) on three cultivars of corn.

a. Fresh Weight (g)

	NK 51036		Bonanza		NC+59	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	2.50±1.39	0.75±0.27	4.22±1.24	1.36±0.44	5.78±1.09	2.90±0.53
SO_2	2.81±1.04	0.62±0.25	3.24±0.78*	1.08±0.25	4.52±1.52*	1.61±0.67*

b. Osmotic Potential (Ψ_π, bar)

	NK 51036		Bonanza		NC+59	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	-6.09±0.22	-3.93±0.36	-6.14±0.20	-4.19±0.35	-7.46±0.55	-3.88±0.63
SO_2	-5.96±0.30	-4.67±0.45*	-5.91±0.38	-4.13±0.71	-6.32±0.34*	-4.15±0.58

c. Leaf Water Potential (Ψ_{leaf}, bar)

	NK 51036	Bonanza	NC+59
Control	-1.17 ± 0.20	-2.71 ± 0.25	-1.50 ± 0.00
SO_2	-1.08 ± 0.25	-1.33 ± 0.26*	-0.92 ± 0.20*

Each number is the average of 12 plants. Asterisk denotes significance difference at the 5% level. Analysis based on paired T-test.

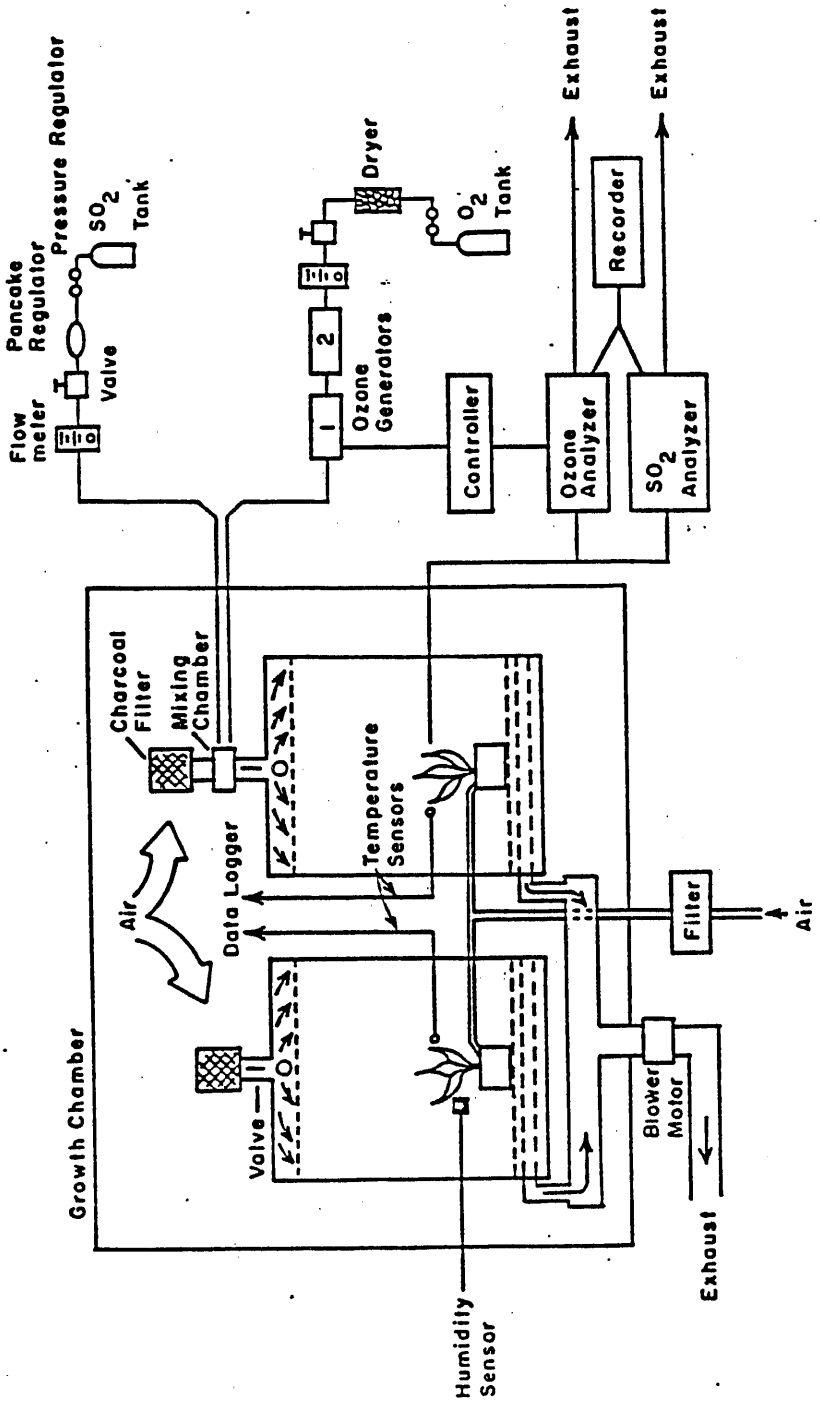

Figure IV.1 Diagram of the fumigation chambers.

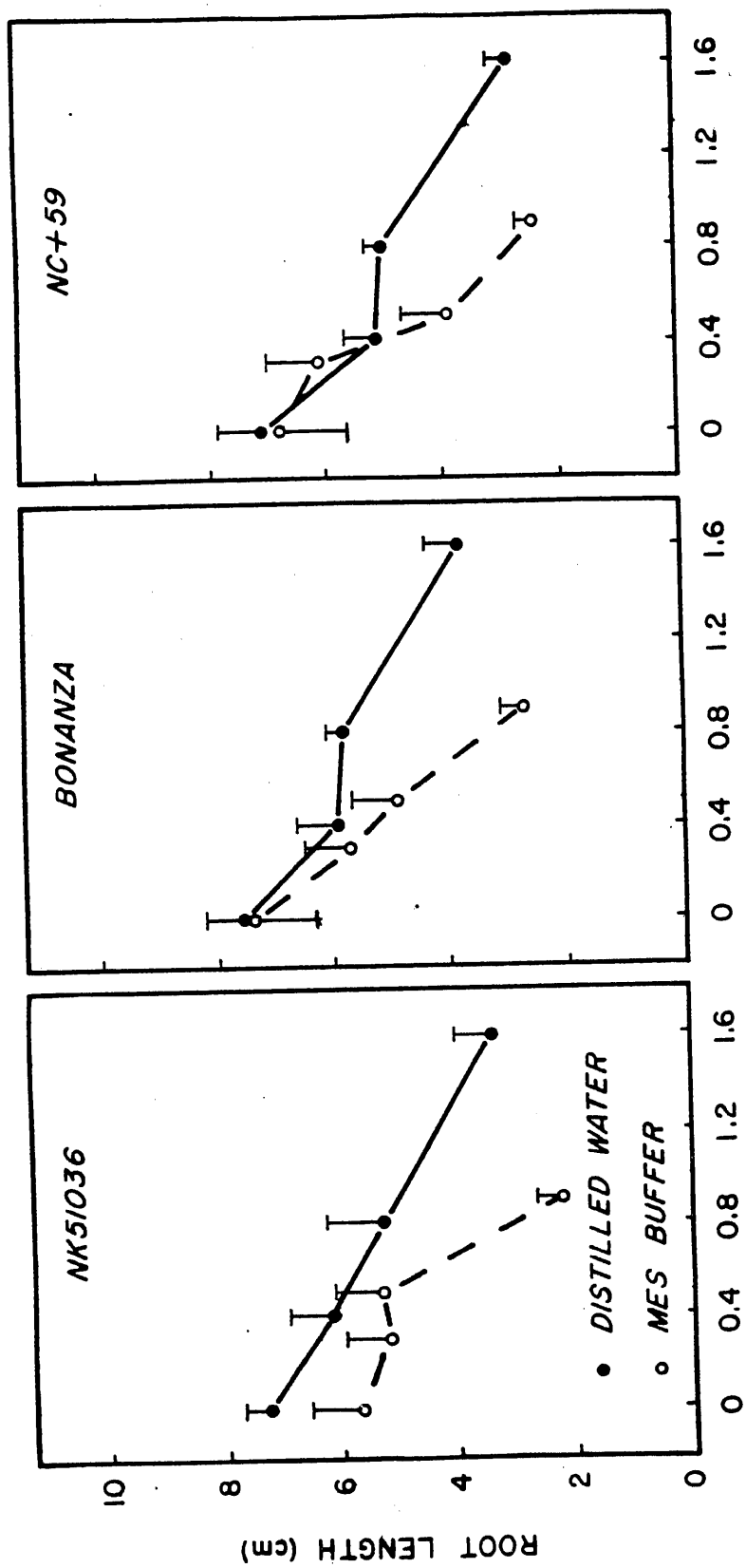

Figure IV.2 The effect of 4 hour daily fumigation with SO_2 for five days at 25°C on root elongation of three cultivars of corn seedlings grown in distilled water and in a MES-buffer medium.

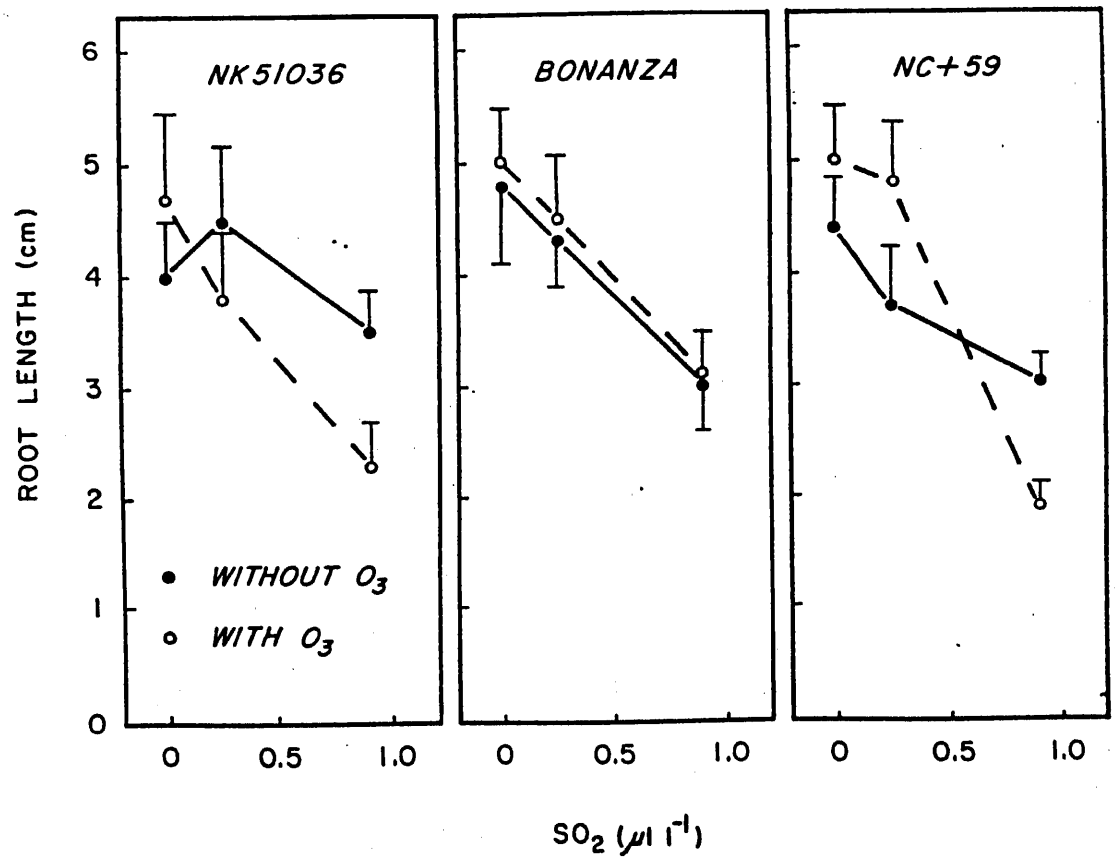

Figure IV.3 The effect of 4 hour daily fumigation with two levels of SO_2, with and without 0.14 $\mu\ell\ \ell^{-1}$ O_3, for 5 days at 25°C on root elongation of three cultivars of corn seedlings.

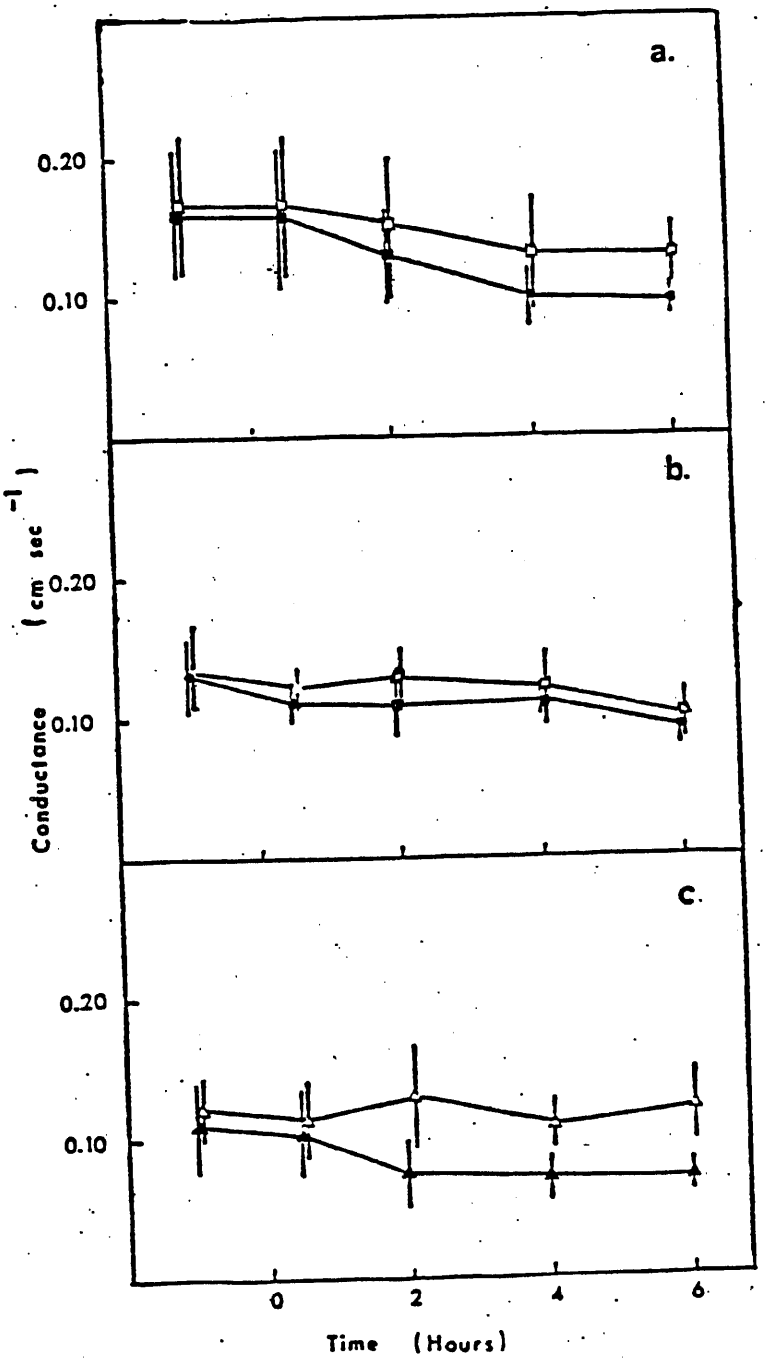

Figure IV.4 Time course of stomatal conductance in three corn cultivars. Time axis indicates hours of SO_2 fumigation at 1.5 $\mu\ell\ \ell^{-1}$. First measurement 1 hour prior to fumigation. Each data point is the mean of eight measurements: a. NC+59 ([] control; ■ SO_2 treated) b. Bonanza (0 control; ● SO_2 treated) c. NK 51036 (Δ control; ▲ SO_2 treated).

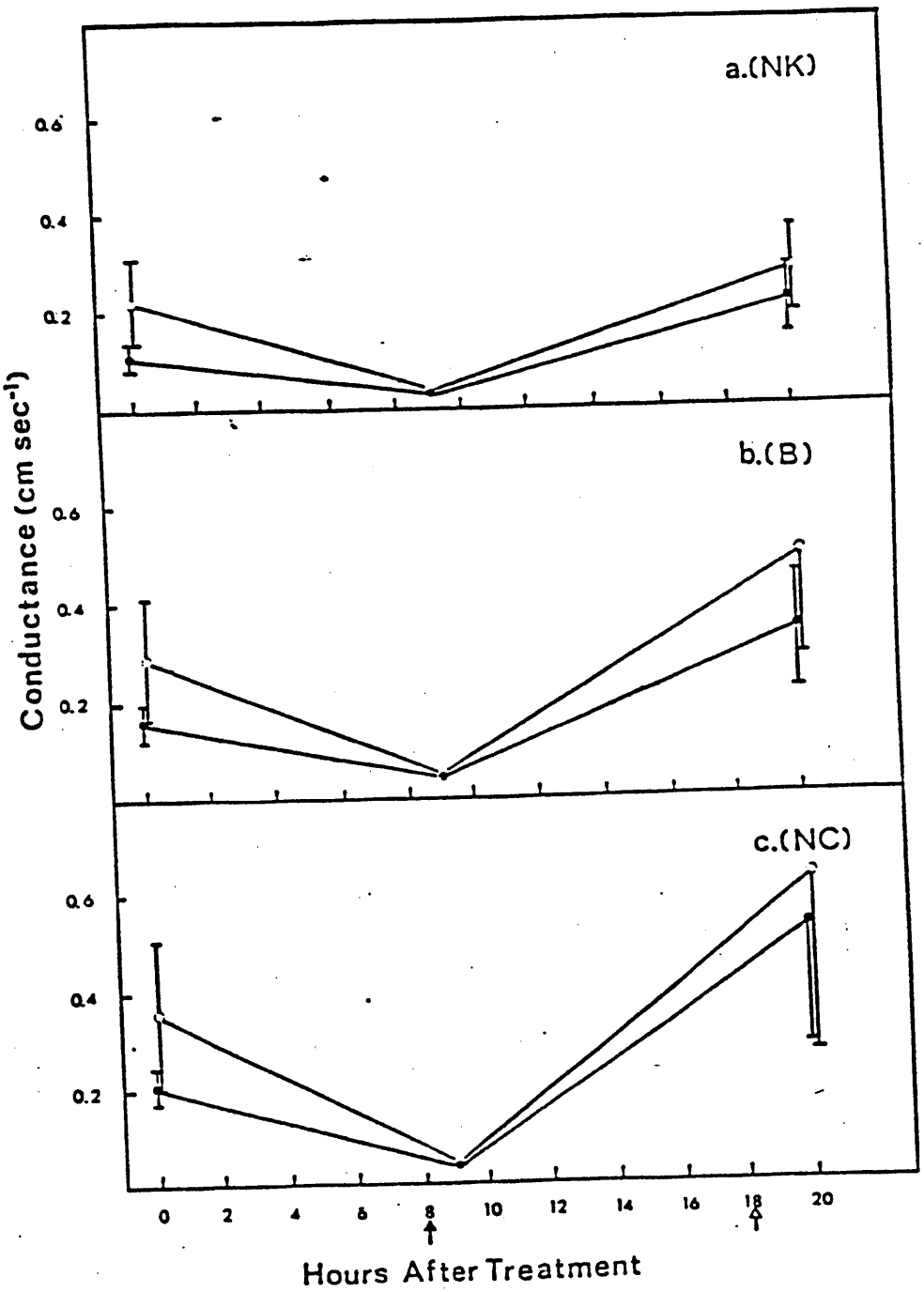

Figure IV.5 Long-term effects of SO_2 on stomatal conductance (0, control; 0, 1.5 μℓ $ℓ^{-1}$ SO_2, 6 hr). Darkened arrow indicates end of photoperiod. Light arrow indicates beginning of next photoperiod (photoperiod for this experiment was set at 14 hr).