SHORT-TERM KINETIC STUDIES ON THE INHIBITION OF PHOTOSYNTHESIS BY SULFUR DIOXIDE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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INTRODUCTION

Sulfur dioxide, a major air pollutant, and its effect on vegetation has been the subject of intensive study for many years. Major reviews by Thomas (43,45), Thomas and Hendricks (46), Daines (13), and Brandt and Heck (9) in America, and by Garber (21,22) in Germany plus minor reviews (14,19,26,44) have adequately summarized these earlier investigations. Recently, publications (7,25,27) containing color photographs of the visible effects of SO_2 (as well as other pollutants) on vegetation have become available to aid in the diagnosis of plant diseases caused by various air pollutants.

The extent of visible injury has been reported to be a function of leaf age, the younger and older leaves being considered more resistant to SO_2 (40,49). The following experiments were designed to explore the possibility that the photosynthetic mechanism of middle-aged leaves may also prove to be more sensitive to SO_2 than that of younger or older leaves. Particular attention has been given to the comparative kinetics of inhibition and recovery of photosynthesis in SO_2-sensitive and SO_2-resistant plants exposed to short, acute treatments of SO_2 in the range of 1 to 5 ppm.

CHAPTER I

METHODS AND MATERIALS

Preparation of Plant Material

Bean seeds, Phaseolus vulgaris L. cv. Pinto, were obtained from local markets, and corn seeds, Zea mays L., were obtained from the Ohio Seed Company, West Jefferson, Ohio. The Pinto bean was selected since earlier published work has shown this plant to be moderately sensitive to a number of pollutants (9). Corn, on the other hand, has been reported to be fairly resistant to SO_2 (7,9,18). Hence, comparative studies could be conducted.

Pinto beans were soaked for 4 to 5 hours in aerated water and sown in a flat containing a vermiculite-perlite-sand mixture (approximately 2:1:2 v/v). The flat was placed in a Sherer-Gillett controlled environment cabinet, Model CEL 512-37, which was maintained at 25°C during the 14-hour photoperiod and 20°C during the 10-hour dark period. Ten 200-w VHO fluorescent powertubes and six 60-w incandescent lamps provided an intensity of about 1400 ft-c 15 cm above the culture containers as measured by a Weston Illumination Meter, Model 756. After 10 days the most advanced seedlings were selected for uniformity and carefully transferred to 1-liter containers containing aerated

Meyer's (35) solution[1]. The seedlings were allowed to remain in the growth cabinet for periods of up to 11 days. The nutrient solution was usually not changed during this time.

Corn seeds were soaked in aerated water for 4 to 5 hours and then placed on moist paper toweling in a closed container held at room temperature. After 4 days, the seedlings were set in "Ethofoam" blocks which were floated on one-half strength, aerated nutrient solution. The seedlings were allowed to develop for 3 days under laboratory conditions before being transferred to 1-liter containers in the growth cabinet. The nutrient solution, however, contained twice as much chelated iron as was given bean. In order to prevent or reduce chlorosis of the leaves, additional chelated iron was added to the nutrient solution when symptoms began to appear. Freshly prepared iron solution (less than 2 weeks old) was essential for best results in preventing chlorosis in corn. There was no chlorosis problem associated with bean.

The primary leaf of bean and the third leaf of corn were used in all SO_2 experiments. The lower lobes and, where necessary, the sides of the bean leaf were trimmed to accommodate the dimensions of the 10 cm x 15 cm x 1.5 cm Plexiglas cuvette. The corn leaf, on the other hand, was fitted into a specially designed Plexiglas cuvette which enclosed a 16-cm zone in the mid-portion of the leaf. Leaf ages were determined in days from planting. In all experiments

[1] $FeCl_3$ was replaced with Sequestrene-Na-Fe to supply 5 parts per million (ppm) Fe.

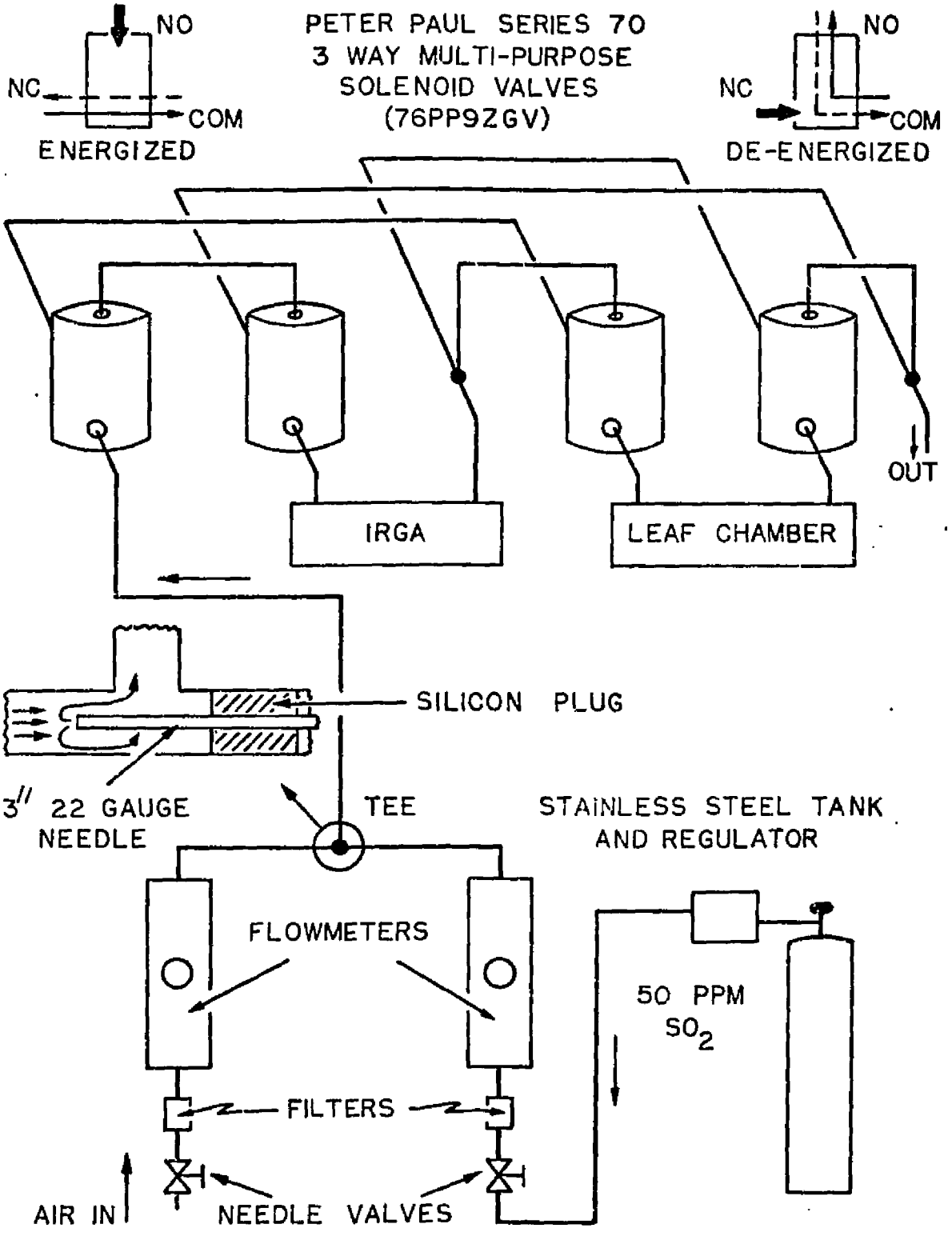
the experimental leaf was placed in the cuvette the night before the experiment was run to allow for equilibration. The 14-hour photoperiod was maintained throughout the equilibration and experimental periods.

Experimental Apparatus

Figure 1 schematically represents the system used in determining net photosynthesis. To avoid excessive pressure drops across the inlet and exit ports of the apparatus, solenoids with 3/16-inch orfice diameters were used. With the solenoids in the de-energized position, compressed air of known CO_2 concentration (varying between 380 and 425 ppm v/v for different tanks) was passed at 1 liter per minute first through the infrared gas analyzer (IRGA) then through the leaf chamber before exiting to the atmosphere. Thus, the CO_2 concentration of the gas stream was determined before entering the leaf chamber. With the solenoids in the energized position the gas was first directed into the leaf chamber and then through the IRGA before exiting to the atmosphere. Therefore, it was possible to determine the difference between the CO_2 concentration of the gas stream entering the cuvette and the CO_2 concentration of the gas stream exiting from the cuvette, the difference being the amount of CO_2 absorbed by the leaf blade, i.e., net photosynthesis.

To provide the desired concentration of SO_2 in air, the standardized SO_2-air mixture (53.2 ppm SO_2 in air, v/v) and air from a compressed air tank were metered through carefully calibrated flowmeters. The respective gas streams were brought together under

Figure 1--Schematic of the apparatus used in measuring net photosynthesis. The mixing apparatus consisted of 2 flowmeters. The flowmeter on the left could deliver a maximum of 1500 ml of air per minute, and the flowmeter on the right could deliver a maximum of 150 ml per minute. A condenser, maintained at 1°C (not shown in the diagram), was attached to the exit port of the leaf chamber to trap water vapor. Solenoid port abbreviations: NC, normally closed; NO, normally open; COM, common. Silicone plug prepared by curing "RTV-11 Liquid Silicone Rubber" purchased from General Electric Company.


NO
NC
COM
ENERGIZED
PETER PAUL SERIES 70
3 WAY MULTI-PURPOSE
SOLENOID VALVES
(76PP9ZGV)
NO
NC
COM
DE-ENERGIZED
OUT
IRGA
LEAF CHAMBER
SILICON PLUG
3" 22 GAUGE
NEEDLE
TEE
STAINLESS STEEL TANK
AND REGULATOR
FLOWMETERS
50 PPM
SO_2
FILTERS
AIR IN
NEEDLE VALVES

conditions of high turbulence in a stainless steel tee fitted with a 3-inch 22-gauge hypodermic needle as shown in Figure 1. Passing the SO_2-air mixture through the small diameter needle increased flow stability as noted by the dampened oscillation of the float.

The respective flow rates for the two gas streams required for a given concentration of SO_2 in air were calculated from the equation: $ppm_1 \times \text{flow rate}_1 = ppm_2 \times \text{flow rate}_2$. Thus for a concentration of 5 ppm:

$$53.2 \text{ ppm} \times Q \text{ ml min}^{-1} = 5 \text{ ppm} \times 1000 \text{ ml min}^{-1}$$

$$Q = \frac{5000 \text{ ml min}^{-1}}{53.2}$$

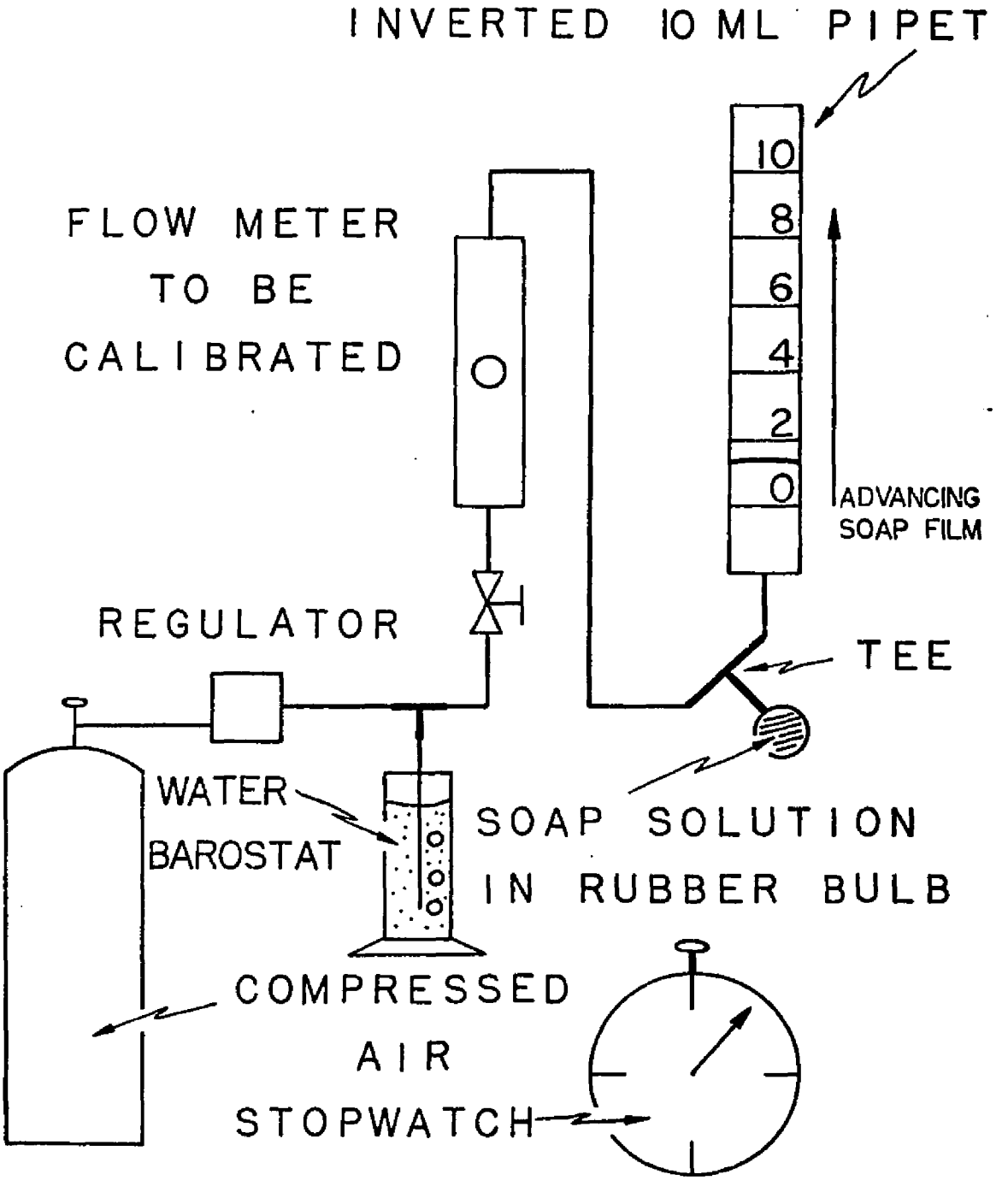
$$Q = 94 \text{ ml min}^{-1}$$

Q is the flow rate in ml min^{-1} of the calibrated SO_2-air mixture. If the SO_2-air mixture at a flow rate of 94 ml min^{-1} is added to an air stream flowing at 906 ml min^{-1}, the combined air streams will have a flow rate of 1000 ml min^{-1} and a SO_2 concentration of 5 ppm v/v. In order to maintain steady flows a fine metering valve (Nupro SS-4MA) was installed on the air input port, and an extra fine metering valve (Nupro SS-2SA) was installed on the SO_2 input port (Figure 1). All filters, needle valves, solenoids, fittings and plumbing except the Tygon leads to the IRGA and Plexiglas cuvette were composed of stainless steel and glass.

Calibration Techniques

The flowmeters were calibrated using the apparatus schematically illustrated in Figure 2. At a set flowmeter reading a soap film was

Figure 2--System for calibrating flowmeters. The water barostat was adjusted to maintain a pressure of 10 cm water above the prevailing ambient pressure.


INVERTED 10 ML PIPET
10
8
6
4
2
0
ADVANCING SOAP FILM
FLOW METER TO BE CALIBRATED
REGULATOR
TEE
WATER
BAROSTAT
SOAP SOLUTION IN RUBBER BULB
COMPRESSED AIR
STOPWATCH

introduced into the pipet, and the time for the film to advance 10 ml was recorded by a stopwatch[2]. The reading was then converted to give milliliters per minute. A calibration curve provided by the manufacturer of the flowmeter, Brooks Instrument Division of Cincinnati, Ohio, and the one generated by the above technique were in close agreement in the 20 to 100 ml per minute range used in the experiments.

Carbon dioxide concentrations were determined using a Mine Safety Appliances, Model 200-LIRA, infrared gas analyzer with a flowing reference cell. Using gases of known concentrations (certified by the manufacturer to be accurate to within $\pm$ 2 percent of the minor component) a calibration curve was generated over the range of 0 to 955 ppm CO_2. From this curve the accuracy of the mixing apparatus was established by mixing various volumes of standard gases and comparing the theoretical values, based on the dilution equation given above, to the measured CO_2 concentration values. As shown in Table 1 the 2 sets of values agreed to within 0.5 percent.

The instrument was spanned over the range of 284 ppm to 420 ppm CO_2. The reference cell was continously purged at 100 ml per minute with a CO_2-N_2 mixture containing 284 ppm CO_2 in nitrogen. To obtain a downscale reading the same gas was passed through the sample cell at 1 liter per minute and the meter reading set to 0

[2]For best results the film or membrane must have sufficient strength to advance through the pipet without rupturing. "Wonder Soap Bubbles" manufactured by Chemtoy Corporation of Chicago and purchased on the local market proved satisfactory for this purpose.

TABLE 1

ESTIMATION OF DILUTION ACCURACY USING INFRARED ANALYSIS TO MEASURE CARBON DIOXIDE CONCENTRATIONS

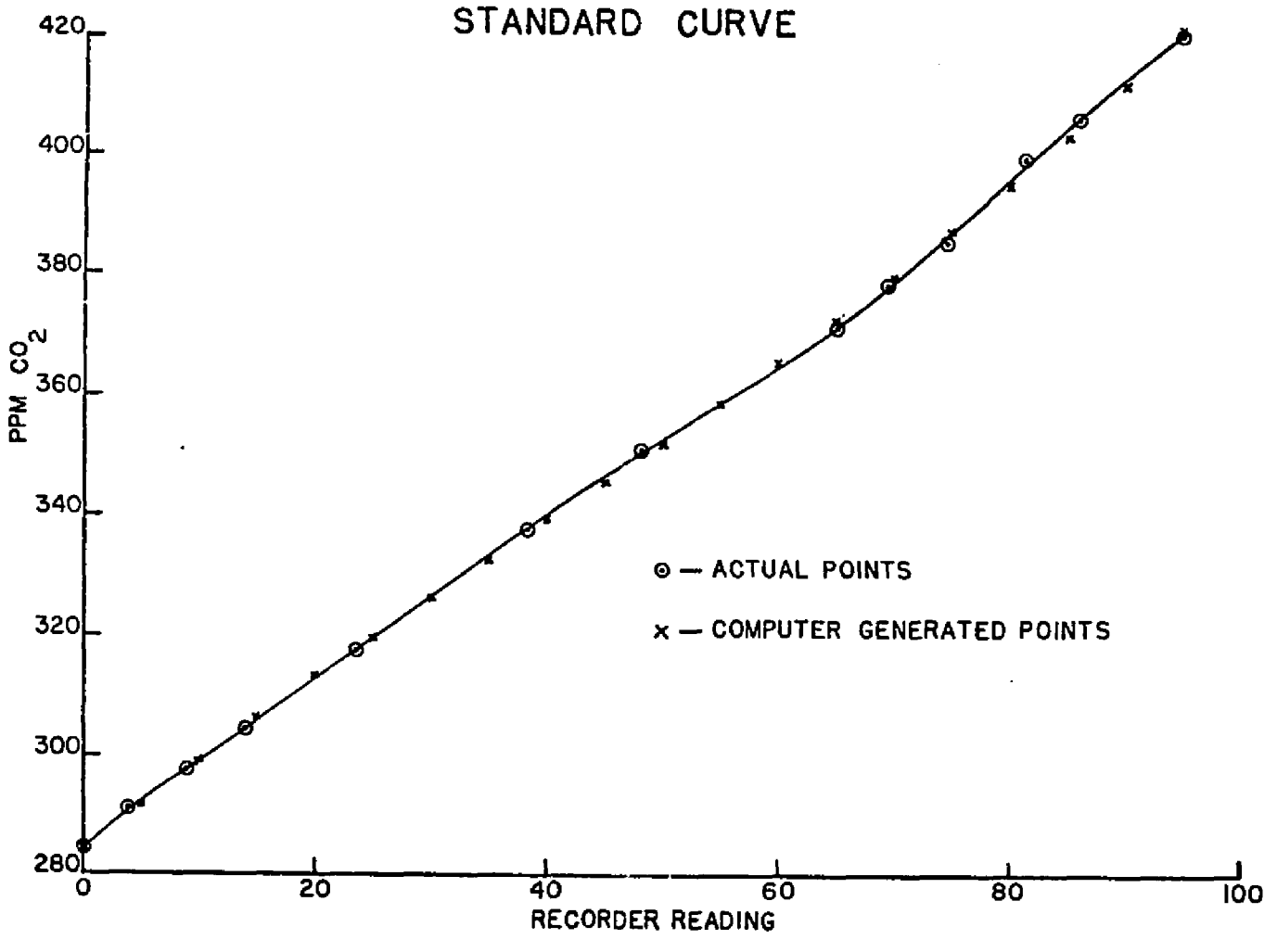
(A) ML MIN^{-1} OF 380 PPM	(B) ML MIN^{-1} OF 955 PPM	(A + B) THEORETICAL PPM	MEASURED PPM
990	10	386	385
980	20	392	390
970	30	397	395
460	40	426	428
950	50	409	408

with the zero adjust control. For an upscale reading a standard gas mixture containing 420 ppm CO_2 in air, purchased from Airco Industries, was passed through the sample cell at 1 liter per minute and the meter set to 95 with the gain and span controls. Therefore, the span from 0 to 95 on the meter corresponded to CO_2 concentrations ranging from 284 ppm to 420 ppm. (The 284 ppm CO_2 to 420 ppm CO_2 span was used since the CO_2 concentration in this range was found to be nonlimiting in bean. This CO_2 range was assumed to be nonlimiting for corn also.)

Intermediate points were generated using the mixing apparatus described above. Known volumes of calibrated gas mixtures of 955, 420 and 284 ppm CO_2 were mixed in varying proportions to give CO_2 concentrations between the lower and upper limits of the curve. A smooth line which best fit the experimental points was manually drawn, and the resultant graph was used as the standard curve in computing photosynthetic rates (Figure 3).

Even though the concentration of CO_2 in the gas stream exiting from the cuvette was monitored continously throughout the 3 to 4 hour experiment, only values taken at 5 minute intervals were actually used in computing photosynthetic rates. To facilitate the handling of these data, an Omnitab program (Appendix A) was written to determine the coefficients of a third degree polynomial which best fit the CO_2 standard curve (second and fourth degree polynomials proved less satisfactory). The curve generated by this equation agreed well with the empirical curve as shown in Figure 3 (cf. Appen-

Figure 3--Comparison of the standard CO_2 curve to the curve generated using a third degree polynomial.


STANDARD CURVE
420
400
380
360
340
320
300
280
PPM CO_2
0
20
40
60
80
100
RECORDER READING
⊙ — ACTUAL POINTS
x — COMPUTER GENERATED POINTS

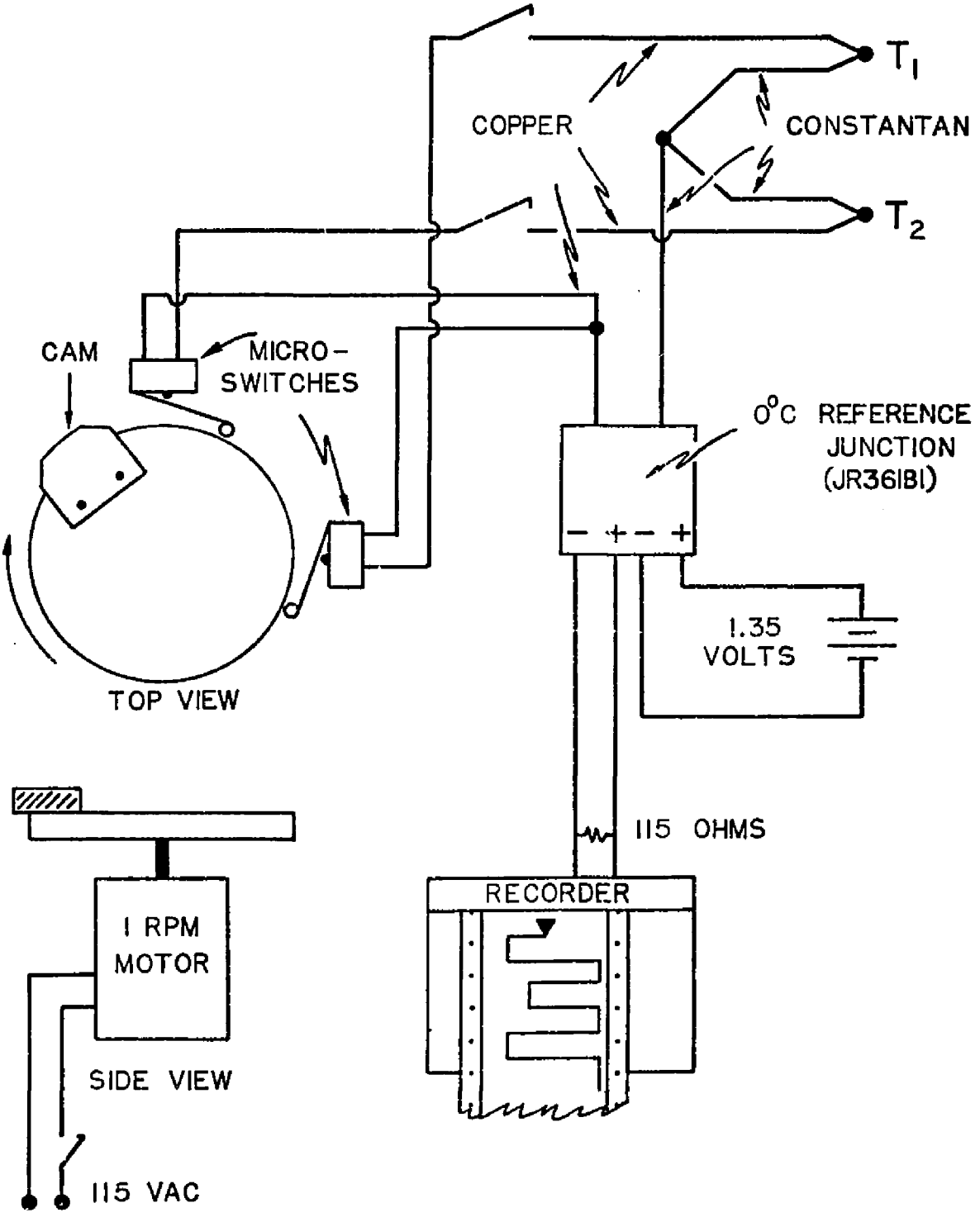
dix B for computer program used to generate curve). It was then possible to enter the recorder readings (IRGA meter readings) into the polynomial and determine CO_2 concentrations mathematically using the computer program given in Appendix C.

Leaf Temperature Determination

Light from two 300-w flood lamps provided an intensity of about 3500 to 4000 ft-c on the upper surface of the leaf cuvette. Preliminary experiments had shown that maximum photosynthetic rates for Black Valentine beans were attained at light intensities of about 2000 ft-c and above. Assuming the light saturation level of Pinto bean to be similar to that of the Black Valentine variety, light intensity was considered nonlimiting in the experiments with Pinto bean. Comparable data for the corn plants used in these experiments were not determined.

In some of the experiments, leaf and air temperatures in the cuvette environment were measured. An automatic switching apparatus was constructed capable of operating 6 independently positioned thermocouples. A diagram of the instrument is given in Figure 4. With this apparatus 1 potentiometric recorder was capable of recording the output from 6 different thermocouples. The scale was determined by immersing the thermocouples in an ice slurry to obtain a zero reading and then into a water bath at a known higher temperature to obtain an upscale deflection. The upscale reading was adjusted on the recorder to give a sensitivity of 4 chart divisions

Figure 4--Schematic of apparatus used in the determination of leaf and air temperatures. Only 2 microswitches are shown for simplification. Reference junction was purchased from Consolidated Ohmic Devices Inc., New York.


T1
COPPER
CONSTANTAN
T2
CAM
MICRO-
SWITCHES
0°C REFERENCE
JUNCTION
(JR36181)
1.35
VOLTS
TOP VIEW
115 OHMS
RECORDER
1 RPM
MOTOR
SIDE VIEW
115 VAC

per degree centigrade. The thermocouple reference junctions, which replaced the conventional ice-water junctions, proved highly satisfactory.

Leaf temperatures ranged from about 28°C to 34°C or about 5°C to 10°C above room temperature. A reduction of about 1°C in the leaf temperature could be effected by circulating air from a small blower over the cuvette, and this procedure was generally adhered to in the later experiments.

Sources of Error

A calibrated gas mixture containing 53.2 ± 2 percent ppm SO_2 (v/v) in air was obtained from Scott Research Laboratories of Plumsteadville, Pennsylvania. The mixture was analyzed by the West and Gaeke method by the supplier and shipped in a stainless steel cylinder (SSB) fitted with a stainless steel regulator (Scott Model 12-660). According to the manufacturer, SO_2 can be adsorbed onto the walls of an ordinary steel cylinder, thereby reducing the SO_2 concentration. In the containers supplied by the manufacturer, the concentration was guaranteed stable at ± 2 percent of the minor component for $1\frac{1}{2}$ years.

Since the infrared absorption bands of water and CO_2 overlap slightly, the interference of water vapor from the transpiring leaf was considered a possible source of error in the determination of CO_2 concentration. In order to maintain a reasonable constant partial pressure of water vapor in the gas stream entering the IRGA,

a condenser (not shown in Figure 1) held at 1°C was attached to the exit port of the cuvette. The concentration of water vapor in equilibrium with liquid water at 1°C caused an apparent increase in the CO_2 concentration of about 6.7 ppm, equivalent to about 4.75 chart units. Therefore, the true concentration of CO_2 exiting the cuvette was 6.7 ppm less than the observed reading.

The condenser served 2 other important roles. First, condensation of water vapor in parts of the system downstream from the condenser was prevented, thus minimizing a potential hazard of liquid water entering the analyzer. Secondly, since the solubility of SO_2 in water is high (22.8 g per 100 ml of water at 0°C [24]), the water trapped out in the condenser served as a sink for the gas. Hence, the concentration of SO_2 in the air passing through the condenser was lowered, reducing possible corrosive damage to the sample cell of the IRGA. A later check revealed no damage to the sample cell.

In addition to permitting rapid determinations of the difference between the concentration of CO_2 in the gas entering and leaving the cuvette, the electrically-operated solenoid circuit also permitted rapid checks on instrumental drift during the experimental period. However, a check for drift was not made during SO_2 treatment since a continuous record of the kinetics of CO_2 inhibition was desired. In usual practice, drift seldom exceeded 1 percent of full scale deflection in 6 hours of operation.

An additional factor influencing the CO_2 readings during the exposure period was the difference in the concentrations of CO_2

between the tank containing compressed air and the tank containing the compressed air plus SO_2. The concentration of CO_2 in the standard SO_2 tank, as measured by the infrared gas analyzer, was 428 ppm (the interference of 50 ppm SO_2 with the determiantion of CO_2 concentration may be disregarded -- personal communication from Mr. G. Zuber, Mine Safety Appliances). Due to mixing of two different gas streams containing unequal concentrations of CO_2, an additional correction factor was included in the computations of net photosynthesis during SO_2 treatment. Because each of the several compressed air tanks used during this investigation contained different CO_2 concentrations, all photosynthetic rate data were normalized to a CO_2 level of 420 ppm v/v, assuming a linear relationship between photosynthetic rate and CO_2 concentration in the CO_2 range used in this study.

Carbon dioxide concentrations in millivolt equivalents or chart units were read from the chart record at 5 minute intervals. Because the flow rate was constant each point represented the average CO_2 concentration of 1 liter of gas which had passed over the leaf during a period of 1 minute. By calculating the milliliters of CO_2 removed from 1 liter of gas, it was possible to derive the CO_2 uptake rate (net photosynthetic rate) in milligrams of CO_2 per hour. The area of the treated primary leaf was determined by planimetry after each experiment and, for comparative purposes, CO_2 uptake data were expressed in units of milligrams per square decimeter per hour. The relative net photosynthetic rates were obtained after SO_2 was introduced into the system. Each rate from time zero was divided by the

rate prior to SO_2 treatment (pretreatment rate) and multiplied by 100 to give the rate of photosynthesis as a percent of the pretreatment rate.

A computer program was developed to incorporate these correction factors into the computation and to plot the relative net photosynthetic rate as a function of time (Appendix C).

Potometer Experiments

A potometer with a capillary arm calibrated in 0.01 ml divisions was employed to determine the effect of SO_2 on water uptake. Water uptake was assumed to be a reasonably instantaneous measure of transpiration (48) and, indirectly, a relative measure of the degree of stomatal opening.

Sixteen- or 17-day-old bean plants were trimmed to 1 primary leaf which was sealed in the cuvette the night before the experiment was run. The roots were set in the potometer well containing approximately 335 ml of aerated nutrient solution. The well was covered with aluminum foil to reduce liquid expansion effects caused by heating from the light sources. About 20 minutes prior to SO_2 treatment, aeration was discontinued and the root system sealed in the well using a split rubber stopper and "Mortite" caulking compound. An air bubble about an inch long was introduced into the capillary. The rate of water uptake was determined 10 to 12 minutes before SO_2 treatment by noting the time for the bubble to move between consecutive 0.01 ml divisions. Usually within 10 minutes steady-state conditions were reached. Water uptake was recorded every 1 to 2 minutes during SO_2

treatment and every 2 to 5 minutes after the SO_2 treatment was terminated. In control experiments, the rate of water uptake remained constant within $\pm$ 10 percent over a time-course of 3 hours.

CHAPTER II

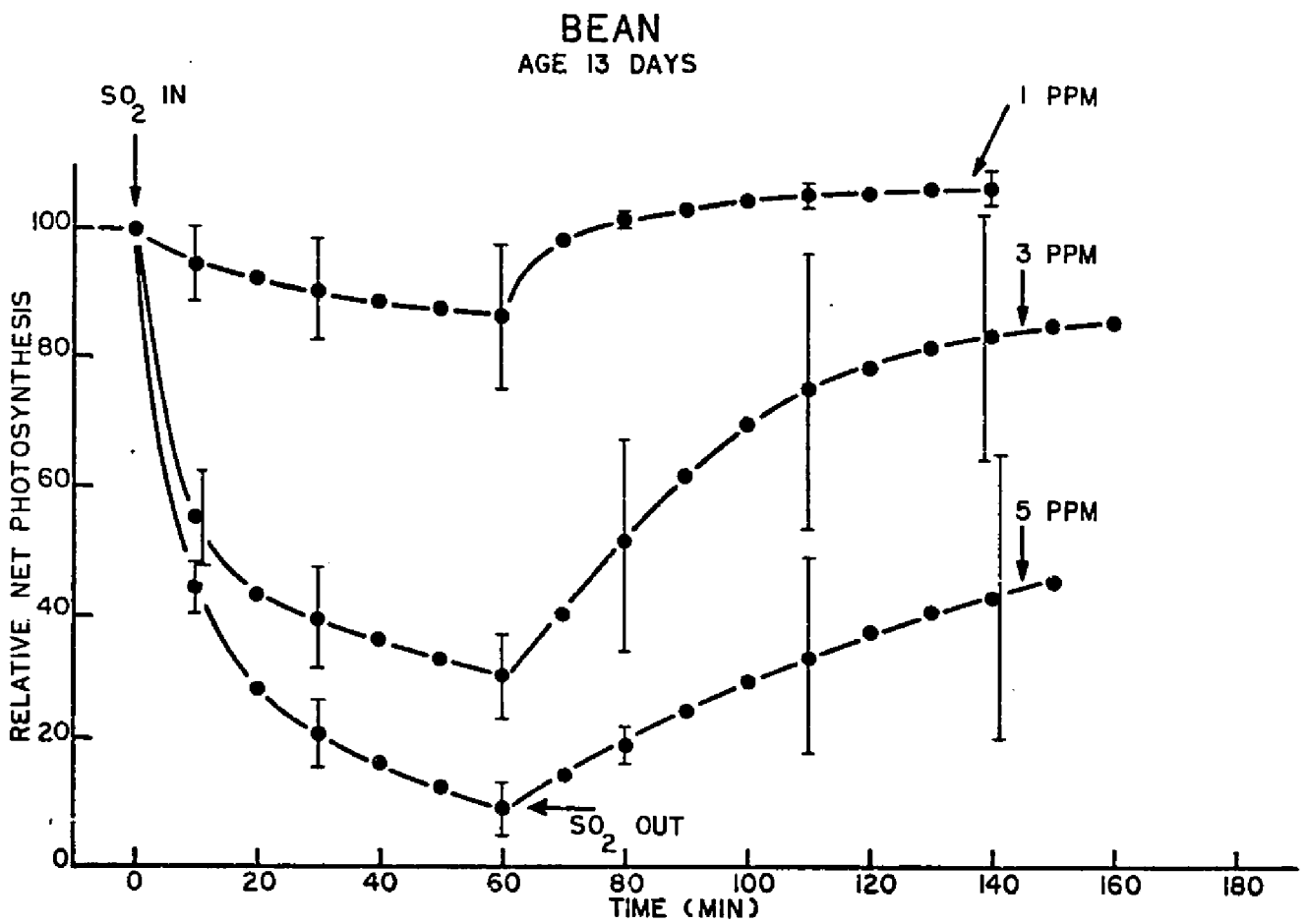
RESULTS AND DISCUSSION

Inhibition of Photosynthesis by SO_2 as a Function of Primary Leaf Age

Primary leaves of 13-, 17- and 21-day-old Pinto beans were subjected to 1, 3 and 5 ppm SO_2 for 1 hour. The net photosynthetic rate was followed continuously both during treatment and 1 to 2 hours after the exposure period. As determined from physical appearances, 13-, 17- and 21-day-old leaves represented young, middle-aged, and old leaves respectively. The 21-day-old leaves usually exhibited visible signs of senescence, i.e., the color of the leaves was a lighter green, and frequently the blades developed yellowish to yellowish-green areas. The respective net photosynthetic rates in mg CO_2 dm^{-2} hr^{-1} for the 13-, 17- and 21-day-old plants used in these experiments measured 19.32 $\pm$ 2.00, 18.54 $\pm$ 3.16, and 11.53 $\pm$ 3.52 (each value is an average of 3 to 4 plants; the range given is $\pm$ 1 standard deviation).

Figure 5 presents the effects of 1-hour treatments with 1, 3 and 5 ppm SO_2 on net photosynthesis in 13-day-old primary leaves of bean. The vertical bars represent $\pm$ 1 standard deviation. A 1-hour treatment with 1 ppm SO_2 decreased the net photosynthetic rate, on the average, about 14 percent. The rate of photosynthesis after

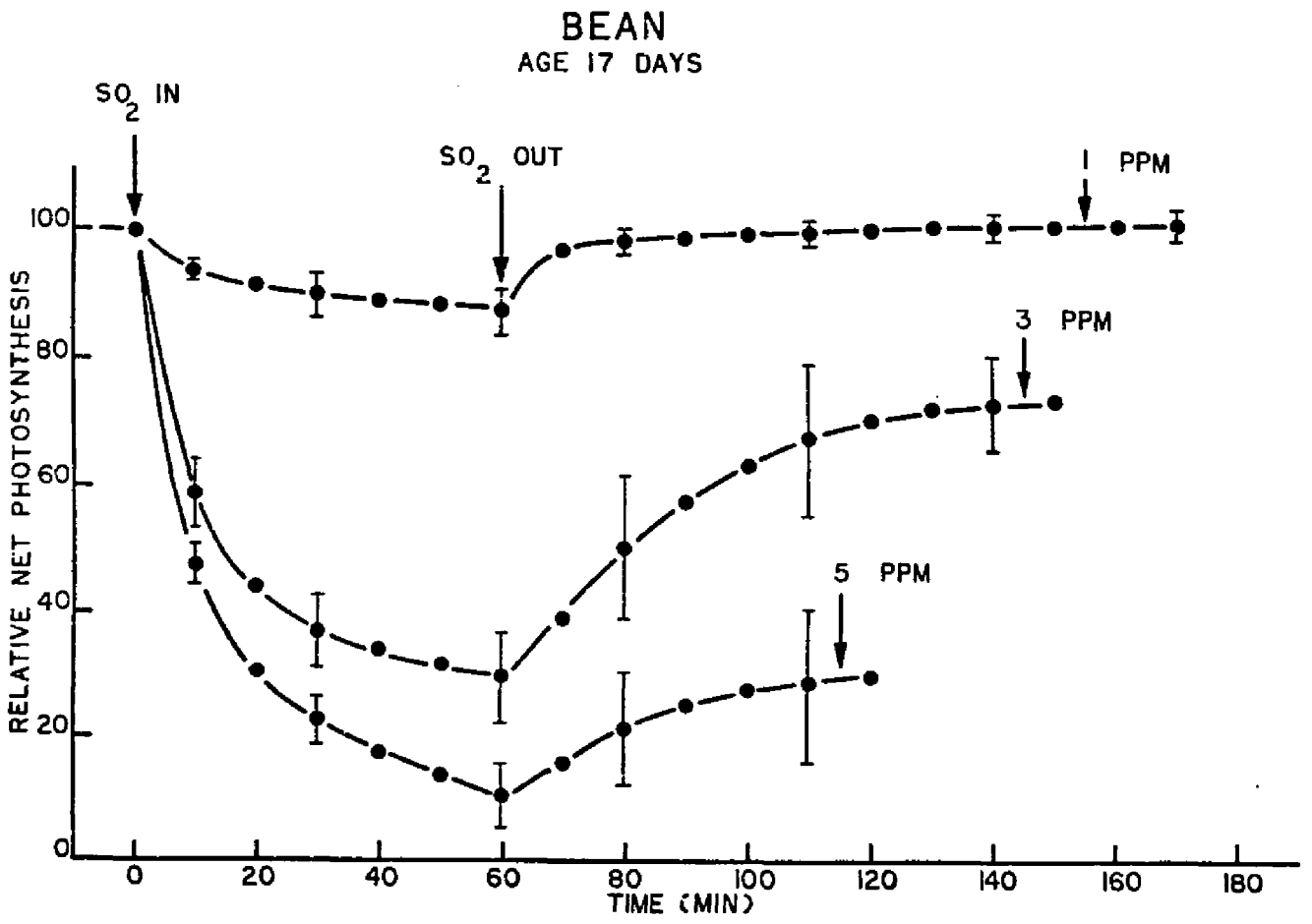
Figure 5--Effects of 1-hour exposures to 1, 3 and 5 ppm SO_2 on net photosynthesis in the primary leaf of 13-day-old Pinto bean. Net photosynthesis was measured as CO_2 uptake. (100 = 19.32 $\pm$ 1.70, 19.55 $\pm$ 1.22, and 19.10 $\pm$ 3.46 $mg\ dm^{-2}\ hr^{-1}$ in the 1, 3 and 5 ppm treated plants respectively.) Each point is an average for 3 to 4 plants.


BEAN
AGE 13 DAYS
SO2 IN
1 PPM
3 PPM
5 PPM
SO2 OUT
RELATIVE NET PHOTOSYNTHESIS
TIME (MIN)
0
20
40
60
80
100
120
140
160
180

recovery from SO_2 treatment, however, reached a value about 6 percent greater than the pretreatment rate. A 1-hour exposure to 1 ppm SO_2, therefore, appears to enhance photosynthesis in 13-day-old leaves for at least a few hours after SO_2 treatment was terminated. Three ppm SO_2 for 1 hour caused a 70 percent reduction in photosynthesis. Two hours after the exposure period photosynthesis recovered to within 85 percent of the pretreatment rate and appeared to be reaching a constant level. Five ppm SO_2 caused the net photosynthetic rate to decrease about 90 percent after 1 hour. Recovery was slower than in the leaves treated with 1 and 3 ppm SO_2 with the rate of photosynthesis reaching about 40 percent of the pretreatment rate 2 hours after removal of SO_2. Recovery was still continuing at this time but at about one-half the initial rate of recovery.

Comparable data for 17-day-old leaves are presented in Figure 6. After 1 hour at 1 ppm SO_2 net photosynthesis was reduced 13 percent but recovered to the pretreatment rate within an hour after SO_2 treatment. No evidence of an enhancement effect was observed in leaves of this age. Three ppm SO_2 reduced the rate of photosynthesis by 70 percent after 1 hour. Within 1 to 2 hours after terminating exposure to SO_2, the photosynthetic rate recovered to 75 percent of the pretreatment rate and, as in the case of the 17-day-old leaves, appeared to be reaching a constant level. A 1-hour exposure to 5 ppm SO_2 reduced photosynthesis 90 percent. The recovered rate 1 hour after removal of SO_2 amounted to only 30 percent of the pretreatment rate and appeared to be nearly constant at this time.

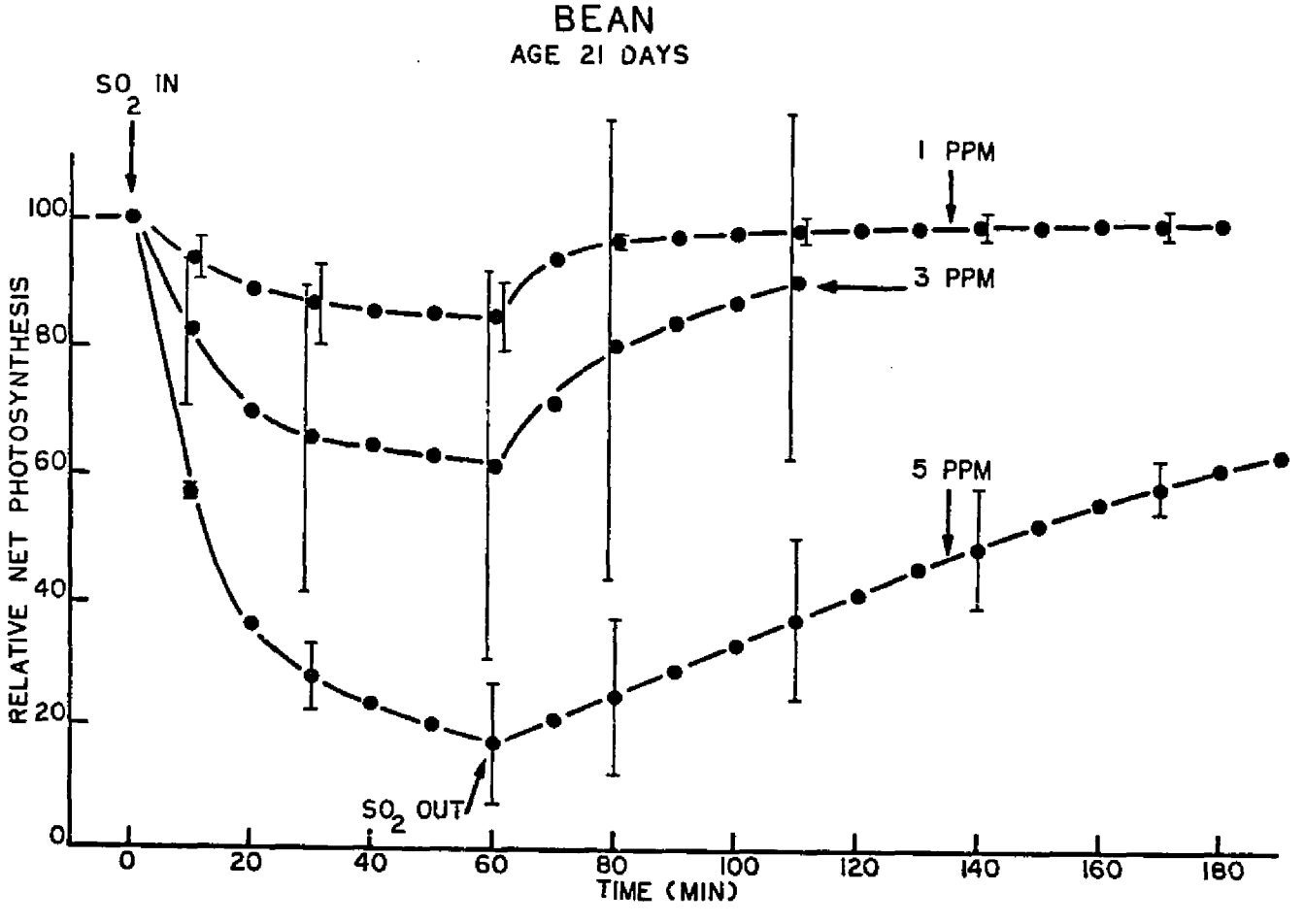
Figure 6--Effects of 1-hour exposures to 1, 3 and 5 ppm SO_2 on net photosynthesis in the primary leaf of 17-day-old Pinto bean. Net photosynthesis was measured as CO_2 uptake. (100 = 19.37 $\pm$ 3.48, 19.38 $\pm$ 1.86, and 16.60 $\pm$ 4.42 mg dm^{-2} hr^{-1} for the 1, 3 and 5 ppm treated plants respectively.) Each point is an average for 3 to 4 plants.


BEAN
AGE 17 DAYS
SO2 IN
SO2 OUT
1 PPM
3 PPM
5 PPM
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
TIME (MIN)

The data for 21-day-old leaves are presented in Figure 7. A 1-hour treatment with 1 ppm SO_2 reduced net photosynthesis by 15 percent. Recovery was complete 1 to 2 hours after terminating exposure to SO_2 with no evidence of an enhancement effect at this time. However, 3 ppm SO_2 decreased photosynthesis, on the average, 40 percent. One hour after the exposure period photosynthesis recovered to within 90 percent of the pretreatment rate. No leveling off of the rate was fully evident within this time, but one may infer by extrapolation of the curve that the fully recovered rate would approach or exceed the pretreatment rate within another hour. At 5 ppm SO_2 the response was similar to that obtained with 13- and 17-day-old leaves, i.e., photosynthesis was decreased 83 percent after the usual 1-hour treatment. Two hours after terminating exposure to SO_2 photosynthesis had reached 62 percent of the pretreatment rate and was still recovering.

In this study, the greatest variability in the net photosynthetic rate occurred in the inhibitory and recovery phases of 21-day-old leaves treated with 3 ppm SO_2. It should be noted that the average net photosynthetic rate of 21-day-old leaves was about one-half the rate of the younger leaves, indicating a significant decrease in the capacity of the older leaves to fix carbon. The greater variability may be attributed to the fact that not all leaves age at the same rate. Hence, the physiological and biochemical processes may vary greatly among plants during senescence. Among the 4 leaves used to obtain data for the graph of the 21-day-old

Figure 7--Effects of 1-hour exposures to 1, 3 and 5 ppm SO_2 on net photosynthesis in the primary leaf of 21-day-old Pinto bean. Net photosynthesis was measured as CO_2 uptake. (100 = 14.79 $\pm$ 3.32, 8.91 $\pm$ 2.91, and 11.76 $\pm$ 1.53 mg dm^{-2} hr^{-1} in the 1, 3 and 5 ppm treated plants respectively.) Each point is an average for 3 to 4 plants.


BEAN
AGE 21 DAYS
SO2 IN
1 PPM
3 PPM
5 PPM
SO2 OUT
RELATIVE NET PHOTOSYNTHESIS
TIME (MIN)
0
20
40
60
80
100
120
140
160
180

leaves treated with 3 ppm SO_2, 1 leaf showed an 84 percent reduction in photosynthesis and recovery to within 85 percent of the pretreatment rate 2 hours after the termination of SO_2 treatment. In the remaining 3 experiments the photosynthetic rate was reduced, on the average, 23 percent after a 1-hour exposure to 3 ppm SO_2. The recovered rates (measured 2 hours after the termination of SO_2 treatment) in 2 of the experiments exceeded the pretreatment rate, one by as much as 14 percent. In these 2 experiments there appeared to be a definite stimulation of CO_2 uptake. The remaining experiment showed recovery of the photosynthetic rate to 95 percent of the pretreatment level.

Net photosynthetic rates in the three 21-day-old leaves which were the least affected by 3 ppm SO_2 were below the average value of 11.53 mg of CO_2 dm^{-2} hr^{-1} which was calculated by averaging the photosynthetic rates of all 21-day-old plants. One could assume that the observed low rates (6.35 to 9.16 mg CO_2 dm^{-2} hr^{-1}) were due to partially closed stomata, and SO_2 may have stimulated them to open (32), allowing greater photosynthetic rates after the SO_2 treatment period. On the other hand, it is possible that photosynthesis was stimulated directly by SO_2. This would have caused the internal CO_2 partial pressure to be lowered in the leaf, thus eliciting an opening response of the stomata. However, no effort was made to determine the diffusion resistances in 21-day-old leaves in order to test the hypothesis that stomata were stimulated to open in the presence of SO_2.

Figures 8, 9 and 10 illustrate the comparative responses of different-aged leaves to a given SO_2 concentration. At 1 ppm (Figure 8) the inhibition of photosynthesis was approximately equal in all leaves tested. The recovered rate was usually highest in the 13-day-old leaves, and, on the average, exceeded the pretreatment rate. The responses of 13- and 17-day-old leaves to 3 ppm SO_2 (Figure 9) were similar during the inhibitory phase, but the younger plants recovered somewhat more rapidly after SO_2 treatment was terminated. The older 21-day-old leaves exposed to 3 ppm SO_2 showed not only less inhibition but also a higher recovery level than either the younger or middle-aged leaves. At 5 ppm SO_2 for 1 hour (Figure 10) leaves of all ages responded similarly to SO_2 treatment. Recovery of photosynthesis was again greater in the 13-day-old and 21-day-old plants; however, it should be noted that the respective rates of net photosynthesis in the different-aged leaves were normalized to 100 to facilitate comparison of the kinetics of inhibition and recovery. The absolute rates for the 21-day-old leaves were actually about 60 percent of those in the 13-day-old leaves.

Preliminary experiments (based on 3 plants, data not shown) with 10-day-old primary bean leaves treated with 3 ppm SO_2 for 1 hour showed inhibition kinetics similar to the older leaves; however, the recovered rate generally appeared to be greater. Both primary leaves of the 10-day-old plants were enclosed in the cuvette and the flow rate reduced to 500 ml min^{-1} in order to obtain measurable CO_2 uptake readings.

Figure 8--Comparison of the relative net photosynthetic rates of 13-, 17- and 21-day-old leaves treated with 1 ppm SO_2 for 1 hour. Standard deviations were omitted for purposes of clarity.

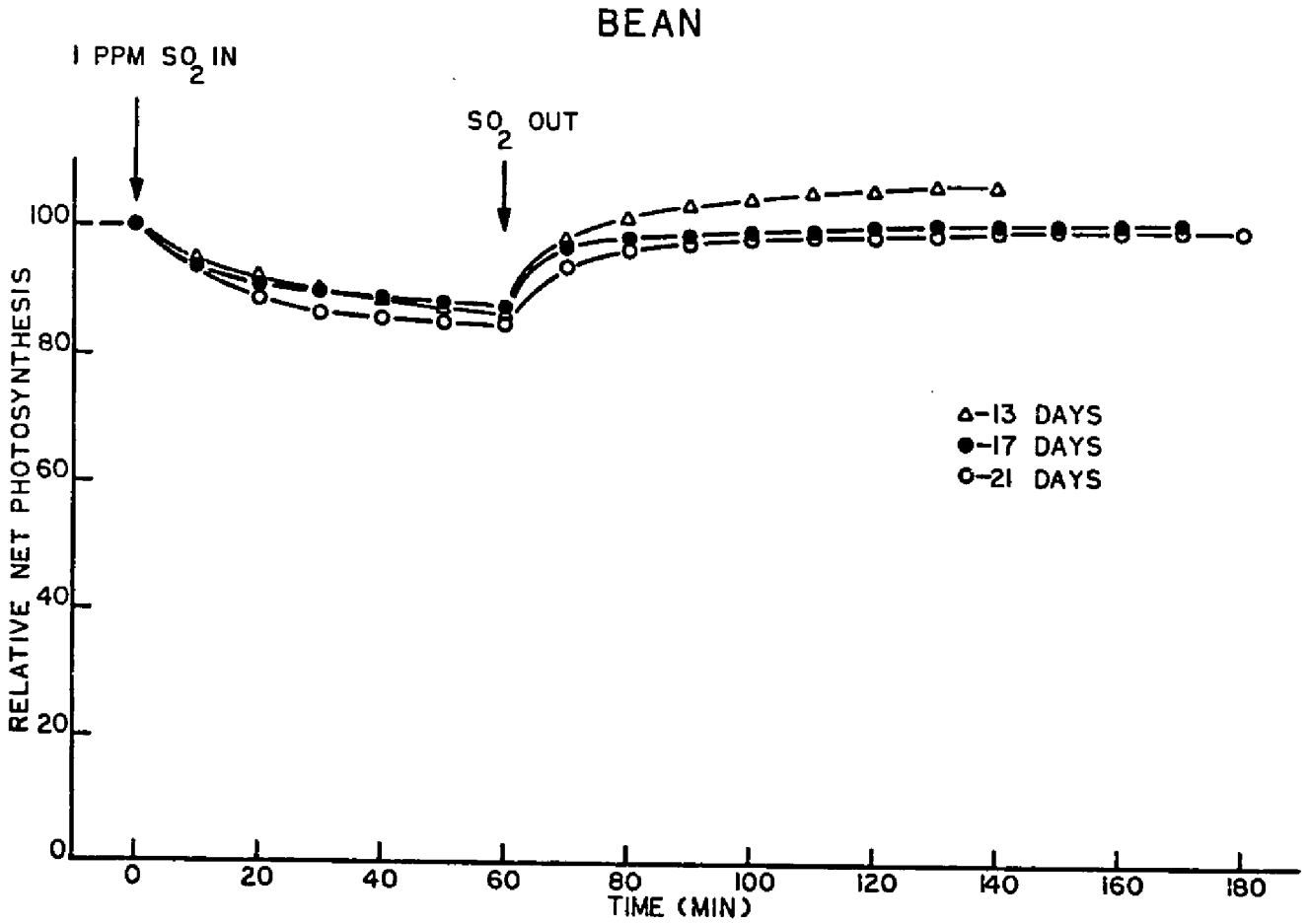

BEAN
1 PPM SO2 IN
SO2 OUT
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
TIME (MIN)
△-13 DAYS
●-17 DAYS
○-21 DAYS

Figure 9--Comparison of the relative net photosynthetic rates of 13-, 17- and 21-day-old leaves treated with 3 ppm SO_2 for 1 hour. Standard deviations were omitted for purposes of clarity.

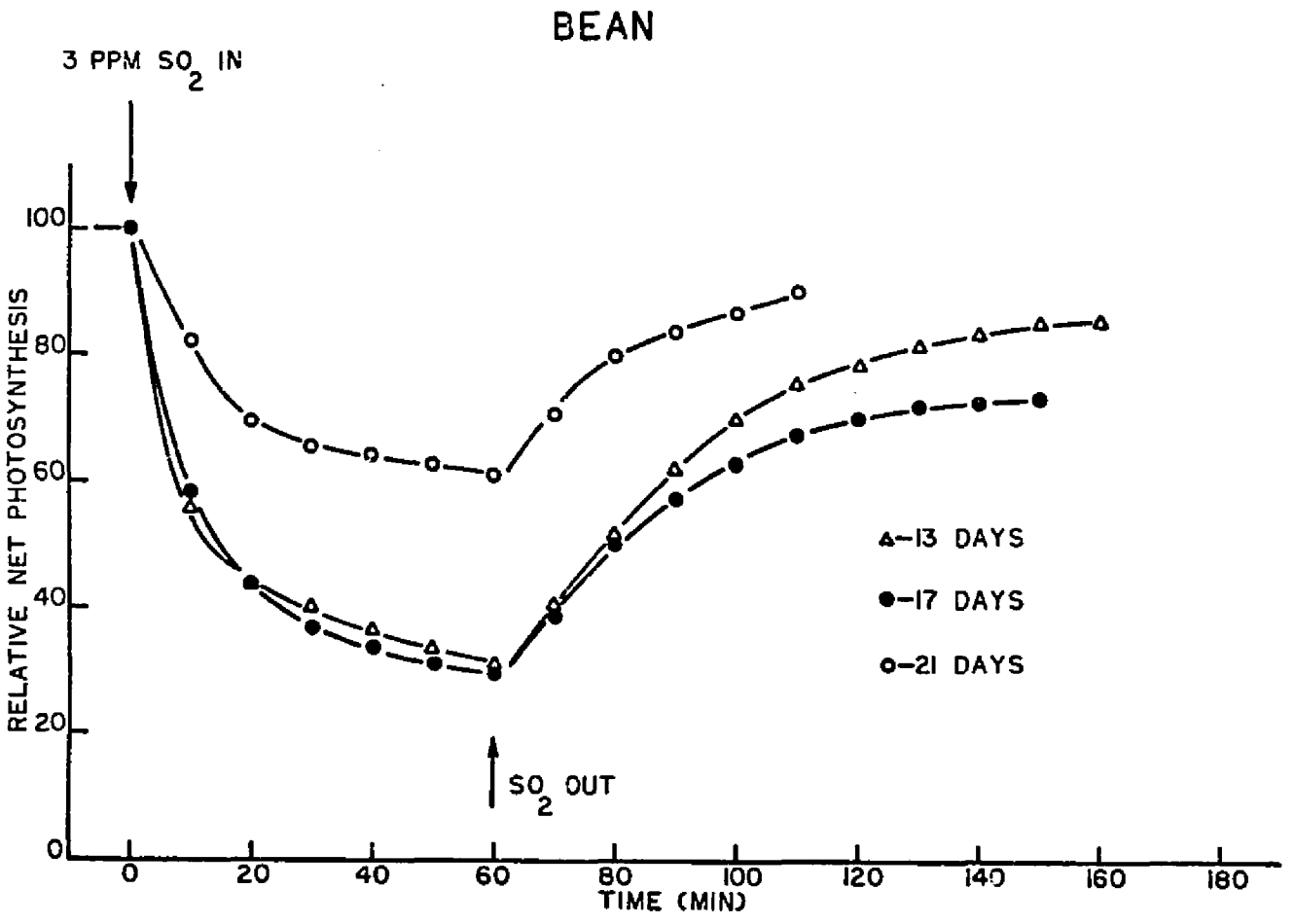
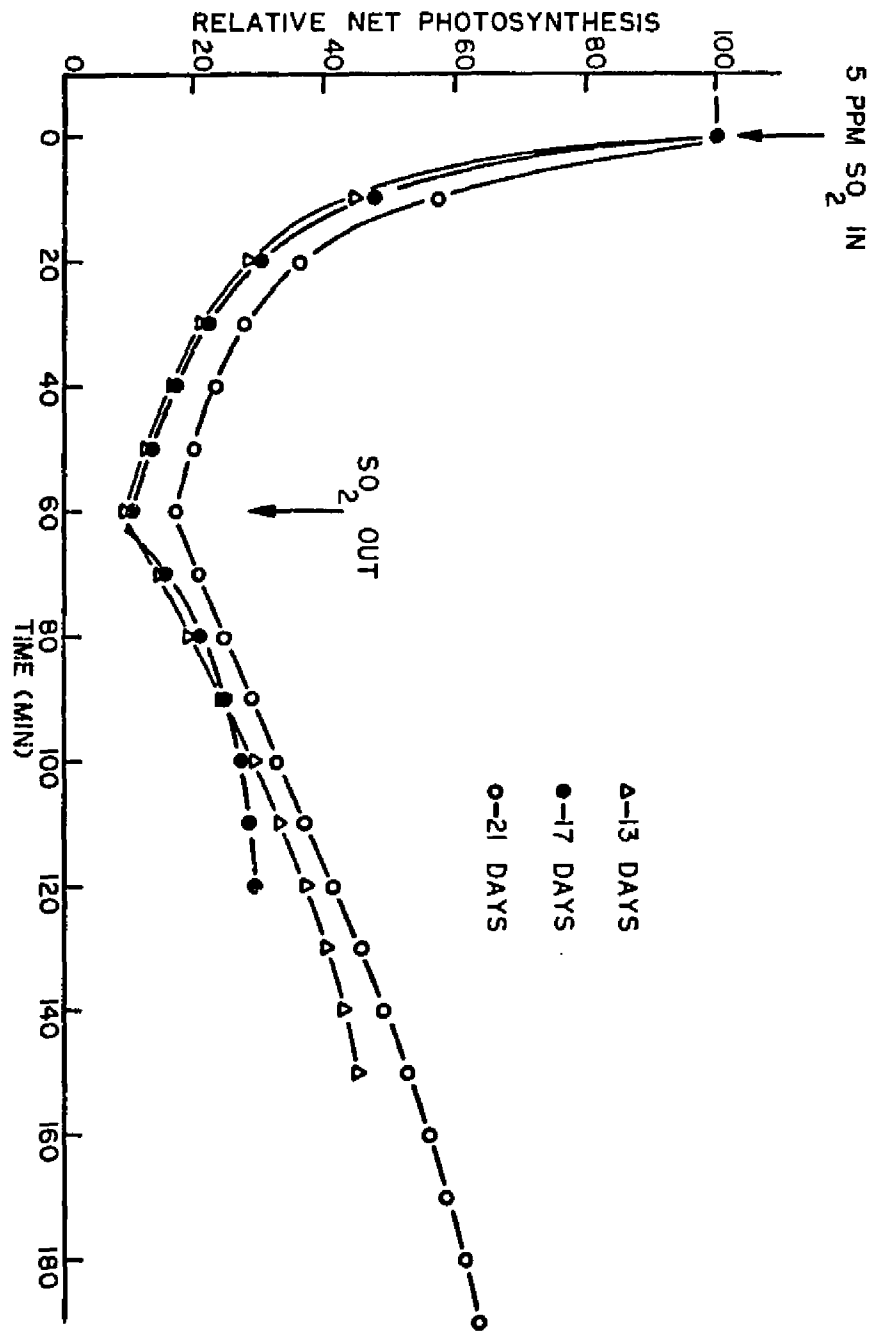

BEAN
3 PPM SO2 IN
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
TIME (MIN)
SO2 OUT
△—13 DAYS
●—17 DAYS
○—21 DAYS

Figure 10--Comparison of the relative net photosynthetic rates of 13-, 17- and 21-day-old leaves treated with 5 ppm SO_2 for 1 hour. Standard deviations were omitted for purposes of clarity.


RELATIVE NET PHOTOSYNTHESIS
0
20
40
60
80
100
5 PPM SO2 IN
SO2 OUT
TIME (MIN)
0
20
40
60
80
100
120
140
160
180
Δ-13 DAYS
●-17 DAYS
o-21 DAYS

BEAN

In summary, it appears that the relative degree of inhibition of photosynthesis by SO_2 is similar in leaves of all ages tested. With the possible exception of 21-day-old leaves treated with 3 ppm SO_2, the resistance of the photosynthetic process to SO_2 inhibition did not appear to be correlated with leaf age. However, the level to which relative net photosynthesis recovered was greater in the younger and older leaves. Thus, as far as the photosynthetic process is concerned it appears that resistance to SO_2 may lie in the greater ability of the younger and older leaves to repair injury to the photosynthetic apparatus during periods of negligible or very low SO_2 concentrations.

Maximum SO_2 levels in 1964 in several large United States cities rarely exceeded 1 ppm SO_2 for 1 hour (42). Consequently, the concentrations employed in the present study, particularly the 3 and 5 ppm levels, represent extreme situations seldom encountered naturally. The concentration levels chosen represent a compromise to permit measurable effects with present techniques over short periods of time. Very high concentrations (10 ppm or greater) were avoided as being too atypical. Although it would obviously be useful to extend these studies to chronic, low level exposure effects (0.5 ppm or less), the present data are useful in suggesting that the susceptibility of the photosynthetic process to SO_2 damage does not vary significantly with leaf age but that the capacity for recovery does.

The data are further significant in showing clearly that net photosynthesis can be severely inhibited in leaves exhibiting no

visible damage. No leaves subjected to 1 ppm SO_2 for 1 hour showed visible injury during the course of the 3- to 4-hour experiment even though net photosynthesis was reduced by as much as 15 percent during treatment. Thomas (47) has shown similar kinetics (inhibition and recovery) with alfalfa using concentrations of 1 ppm SO_2 or less. However, his exposure times were longer, and short-term kinetic studies involving specific leaf ages were not undertaken at higher SO_2 levels.

Similarly, 3 ppm SO_2 for 1 hour caused no noticeable injury within the time-course of these experiments, yet photosynthesis was reduced 70 percent in 13- and 17-day-old leaves. More significantly, the recovered rate 1 to 2 hours after terminating exposure to SO_2 had very nearly reached constant values considerably below the pretreatment levels. There appeared to be a 14 percent and a 25 percent reduction in the photosynthetic capacity for 13- and 17-day-old leaves respectively, and yet no visible injury was evident several hours after treatment.

At 5 ppm SO_2 symptoms typical of acute injury (7) were noted in many but not all leaves. In the affected leaves some of the intervenal tissue appeared collapsed having a dull, grayish-green color. Most of this injury disappeared within 3 to 24 hours after terminating exposure to SO_2, i.e , many of the collapsed areas fully regained their turgor and normal color. Some areas of the leaf (visually estimated to be less than 5 percent of the leaf area) were permanently damaged. This observation supports the conclusion based on kinetic data that leaves have considerable capacity for repair of damage,

depending, of course, on the exposure level.

It was concluded from these experiments that the photosynthetic capacity of a leaf may be impaired, at least temporarily, much more than visible injury would indicate, and, more importantly, that there could be a significant reduction (possibly permanent) in photosynthesis with little or no visible injury. Hence, one may expect a reduction in plant growth and vigor without visible signs of injury. Bleasdale (8) has shown that suppression of growth in S23 ryegrass without visible injury can occur in a polluted atmosphere (containing 0.01 to 0.06 ppm SO_2 as well as other unidentified pollutants). Perhaps a reduced level of CO_2 fixation was the primary limiting factor. Further studies involving longer recovery periods are needed.

As indicated by the standard deviations, leaves treated with 3 or 5 ppm SO_2 showed greater variability in the recovery phase than in the inhibitory phase. At 1 ppm SO_2 the reverse appeared true. Although a part of this variability perhaps reflects minor differences in the prior history of the test leaves, the genetic variability wihtin the population is undoubtedly a very significant factor. Wide variability in sensitivity to SO_2 is known to exist in various species. In white pine, for example, certain individual trees are visibly injured at SO_2 dosage levels as low as 0.05 ppm for 1 hour (12); other individuals tolerate dosages as high as 0.25 ppm for several hours (31). Screening programs to select for genetic resistance to SO_2 and other atmospheric pollutants should be implemented.

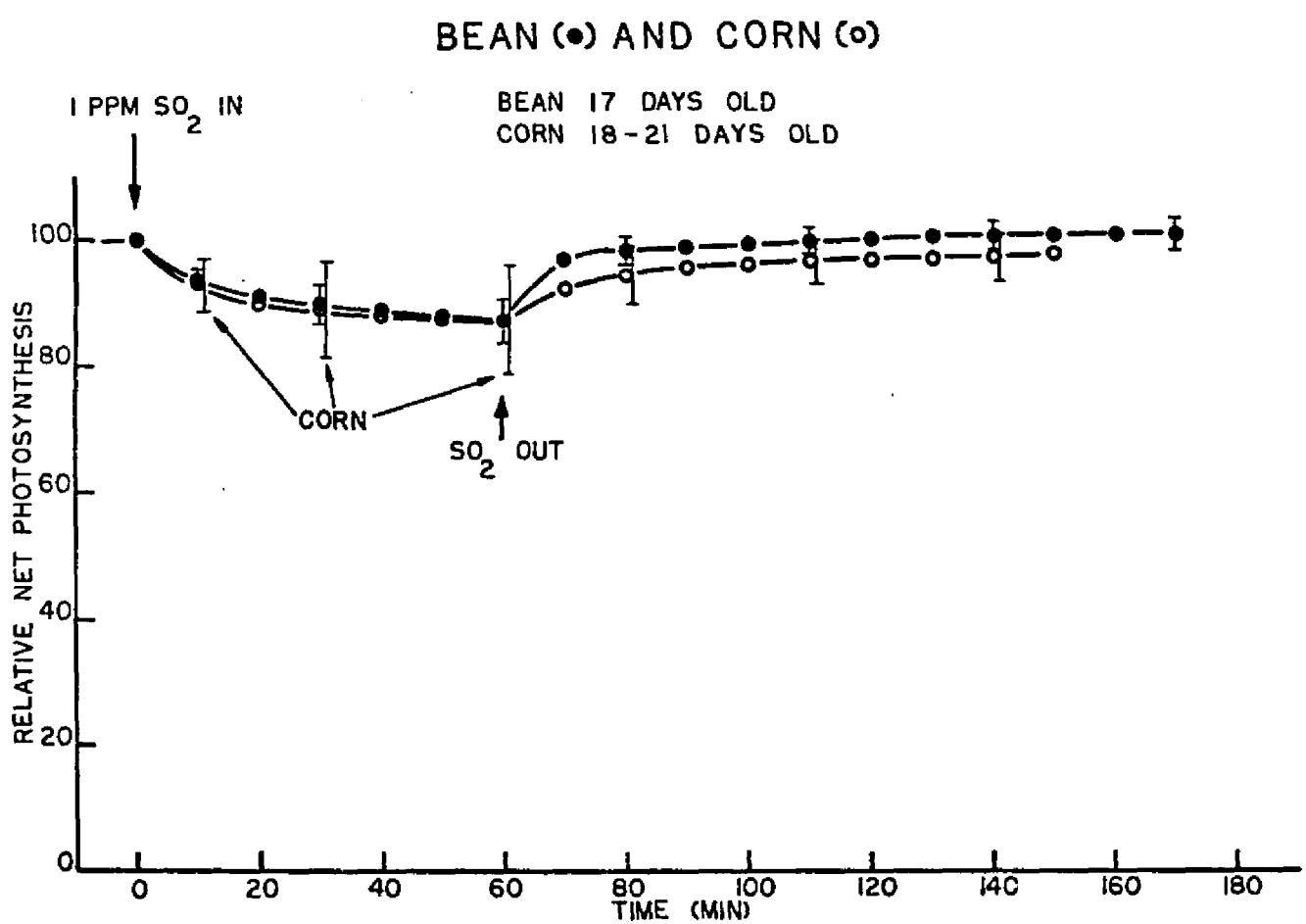
In urban or industrial areas, various atmospheric pollutants usually occur simultaneously with SO_2, and these may act synergistically with SO_2 (15,29,34). For example, Dochinger and Seliskar (15) showed that the visible injury to the leaves of eastern white pine exposed to 0.1 ppm each of ozone and sulfur dioxide was 4 to 5 times greater than the visible injury caused by either pollutant alone at this concentration. Menser and Heggestad (34) showed comparable data for tobacco. A comparative study of the kinetics of inhibition and recovery of photosynthesis in response to various combinations and concentrations of atmospheric pollutants would be of great interest and would be readily feasible with the equipment developed for the current studies.

Comparative Studies with Corn and Bean

As stated earlier, corn has been reported to be resistant to SO_2, whereas bean has been shown to be moderately sensitive to SO_2. This distinction was based on the extent of visible injury when the plants were exposed to a given concentration of SO_2. Since these species differ greatly in their responses to SO_2 on a morphological level, it was of interest to determine if they differ similarly at the physiological level. Hence, comparative studies on the effect of SO_2 on photosynthesis were undertaken. For purposes of this study, the third leaf of 17- to 21-day-old corn plants was used, the leaves appearing to be in their physiological prime at this age.

As shown by Figure 11 the effects of 1 ppm SO_2 on bean and on corn were virtually identical. In each species the rate of photosynthesis decreased, on the average, 13 percent but recovered 98

Figure 11--Comparison of net photosynthetic rates of bean and corn during and after a 1-hour exposure to 1 ppm SO_2 (for bean, 100 = 19.37 ± 3.48 mg CO_2 dm^{-2} hr^{-1}; for corn, 100 = 39.39 ± 2.57 mg CO_2 dm^{-2} hr^{-1}). Data for beans taken from Figure 6. Each point is an average of 3 to 4 plants.

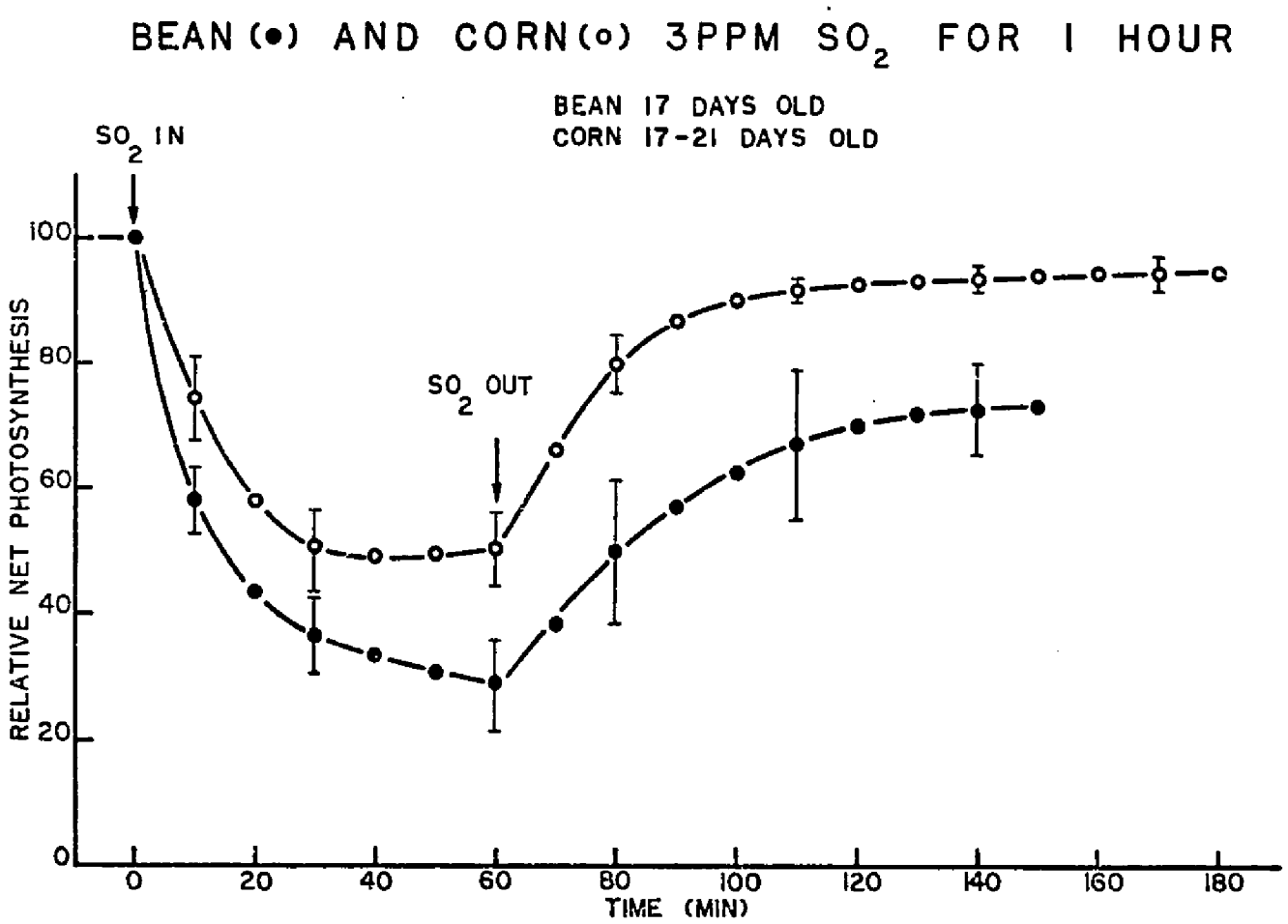

BEAN (●) AND CORN (○)
1 PPM SO_2 IN
BEAN 17 DAYS OLD
CORN 18-21 DAYS OLD
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
CORN
SO_2 OUT
0
20
40
60
80
100
120
140
160
180
TIME (MIN)

to 100 percent of the pretreatment rate 1 to 2 hours after the treatment period. There was no evidence of visible injury in either corn or bean several hours after the exposure period. It was, therefore, concluded from these data that there could be a considerable reduction in the amount of carbon fixed when either "resistant" or "susceptible" species are grown in an atmosphere containing SO_2.

Experiments were then conducted to determine any differences in relative net photosynthesis between bean and corn when leaves were exposed to higher SO_2 concentrations. Figure 12 shows the effects of a 1-hour exposure to 3 ppm SO_2. In corn, even though net photosynthesis was reduced to about 50 percent of the pretreatment rate within the first 30 minutes of exposure, no further reduction was observed during the remaining 30 minutes. In bean net photosynthesis was reduced 60 percent during the first 30 minutes of exposure. During the remaining 30 minutes a more gradual decline in photosynthesis (amounting to about 10 percent) was noted. In both species net photosynthesis recovered about 45 percent. However, because the decline of photosynthesis in corn had reached a constant level after 30 minutes, the overall recovery approached 95 percent of the pretreatment rate. In bean recovery approached 75 percent of the pretreatment rate.

On the basis of data presented in Figure 12, it can be concluded that corn has some protective mechanism allowing nearly complete recovery of photosynthesis upon removal of SO_2. In bean a similar mechanism may be present, but its protective capacity may be exceeded during a 1-hour exposure to 3 ppm SO_2. It should be pointed

Figure 12--Comparison of net photosynthetic rates of bean and corn during and after a 1-hour exposure to 3 ppm SO_2 (for bean, 100 = 19.38 $\pm$ 1.86 mg CO_2 dm^{-2} hr^{-1}; for corn, 100 = 39.92 $\pm$ 1.38 mg $CO_2 dm^{-2}$ hr^{-1}). Data for beans taken from Figure 6. Each point is an average of 3 to 4 plants.


BEAN (•) AND CORN (o) 3PPM SO_2 FOR 1 HOUR
BEAN 17 DAYS OLD
CORN 17-21 DAYS OLD
SO_2 IN
SO_2 OUT
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
TIME (MIN)

out that neither corn nor bean showed visible injury during or after SO_2 treatment, although in bean some physiological injury to the photosynthetic process is indicated by the poor recovery in the photosynthetic rate after removal of SO_2. Experiments employing longer recovery times are required to determine whether or not photosynthesis in bean was permanently reduced.

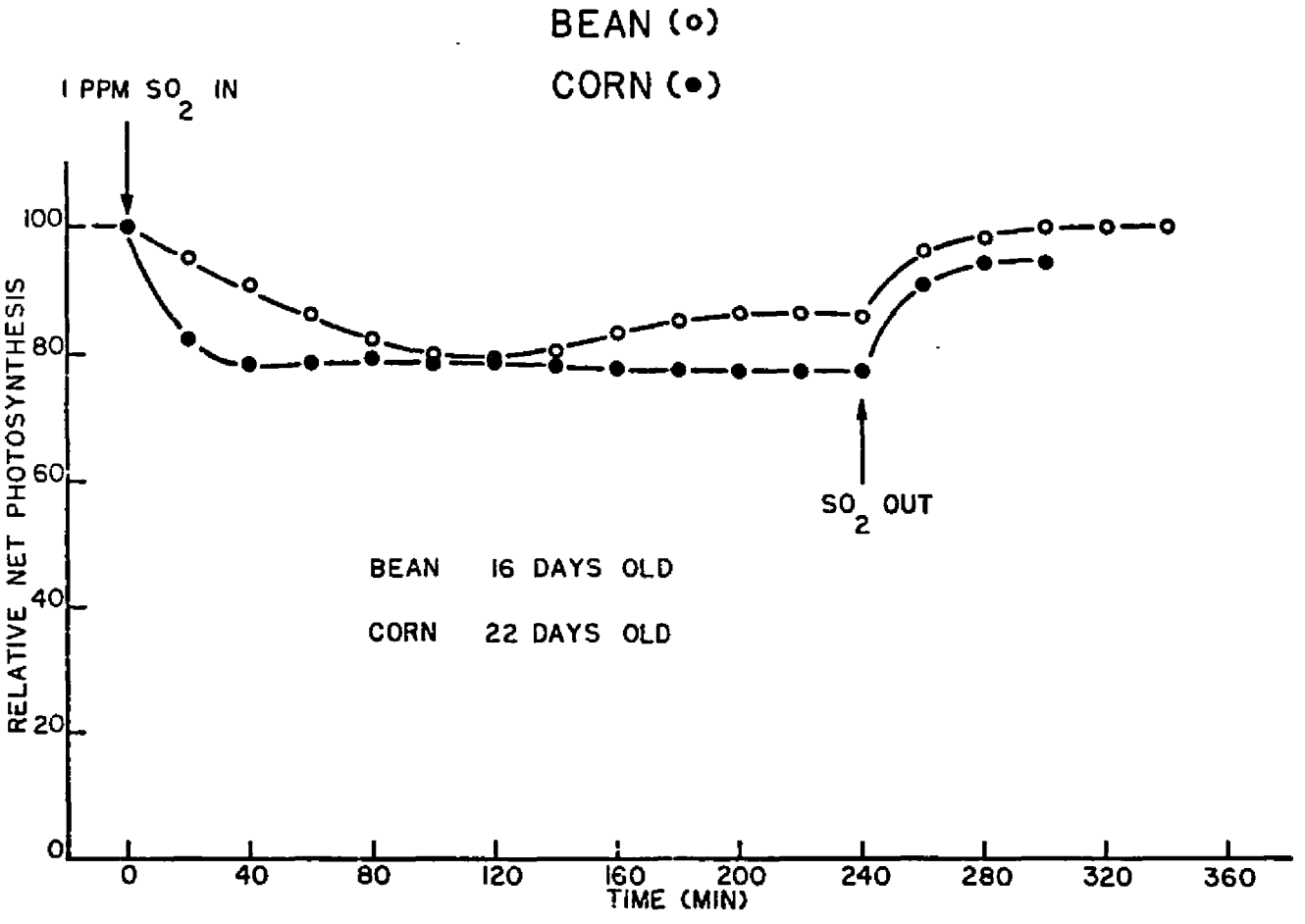
It should be noted that many workers judge the "sensitivity" of a plant to SO_2 by the extent of visible injury after exposure to a given concentration for a given period of time. To achieve visible injury a series of degradative reactions must occur. These reactions are controlled by internal factors or processes that act to oppose those reactions leading to cell destruction. One such opposing process could be the rate at which SO_2 is detoxified to the less toxic sulfate form (45). According to Thomas (45) the sulfite ion (the solution product of SO_2 [9]), because of its high reducing potential, is 30 times more toxic than the sulfate ion. The greater the capacity for detoxification, the smaller the probability that visible injury would develop. There is also a limit to the level to which sulfate may accumulate in cells. Prolonged exposure to low concentrations of SO_2 can eventually cause death of the cells due to the accumulation of sulfate to toxic levels (7).

It would be of interest to extend the period of exposure to 3 ppm SO_2 in order to determine the length of time the rate of photosynthesis in corn could remain at a reduced but constant level and still show a significant recovery of photosynthesis once SO_2 is removed. By treating beans in a manner similar to

that for corn and by comparing the recovery kinetics of both, one could obtain some indication at the physiological level of the relative resistance of each species to SO_2. What this resistance involves is an open question. As indicated above it could involve sulfite oxidation. Resistance might also depend on the presence of an enzyme system capable of repairing damage caused by SO_2. It has been shown that after several hours of photosynthesis leaves with high carbohydrate content were less susceptible to SO_2 (45). Knowledge along these lines could lead to the selection of resistant strains on a biochemical basis.

Although 3 ppm SO_2 is a high concentration, it could be employed to enable selection of the most resistant strains. One ppm SO_2 may be too low to be used in the selection of resistant strains on the basis of short-term physiological effects. Figure 13, based on a single experiment each with corn and bean, tentatively supports this reasoning. The leaves were exposed to 1 ppm SO_2 for 4 hours followed by a 1 to 2 hour recovery period. In both plants the photosynthetic rate was reduced about 20 percent after the first 2 hours of exposure. However, the inhibition of photosynthesis during the first hour of exposure was more gradual in bean than in corn. Photosynthesis in corn reached a constant value of 20 percent below the pretreatment rate and remained essentially at this level for the remainder of the exposure period. In bean after 2 hours of SO_2 treatment, the photosynthetic rate increased from 84 percent of the pretreatment rate to a constant value of about 86 percent of the pretreatment rate. Recovery of photosynthesis was nearly 100 percent

Figure 13--Comparison of the relative net photosynthetic rates of bean and corn during and after a 4-hour exposure to 1 ppm SO_2 (for bean, 100 = 21.40 mg CO_2 dm^{-2} hr^{-1}; for corn, 100 = 36.41 mg CO_2 dm^{-2} hr^{-1}). Each point represents one plant.


BEAN (o)
CORN (•)
1 PPM SO2 IN
SO2 OUT
BEAN 16 DAYS OLD
CORN 22 DAYS OLD
RELATIVE NET PHOTOSYNTHESIS
100
80
60
40
20
0
0
40
80
120
160
200
240
280
320
360
TIME (MIN)

for bean and 95 percent for corn. No visible injury was observed in either plant. In fact, no visible injury had developed in bean 3 days after the completion of the experiment. No record was kept for corn. If there were some mechanism preventing further degenerative changes in the photosynthetic process, then its capacity to restore the photosynthetic process to the pretreatment level after SO_2 was removed must not have been exceeded in either bean or corn.

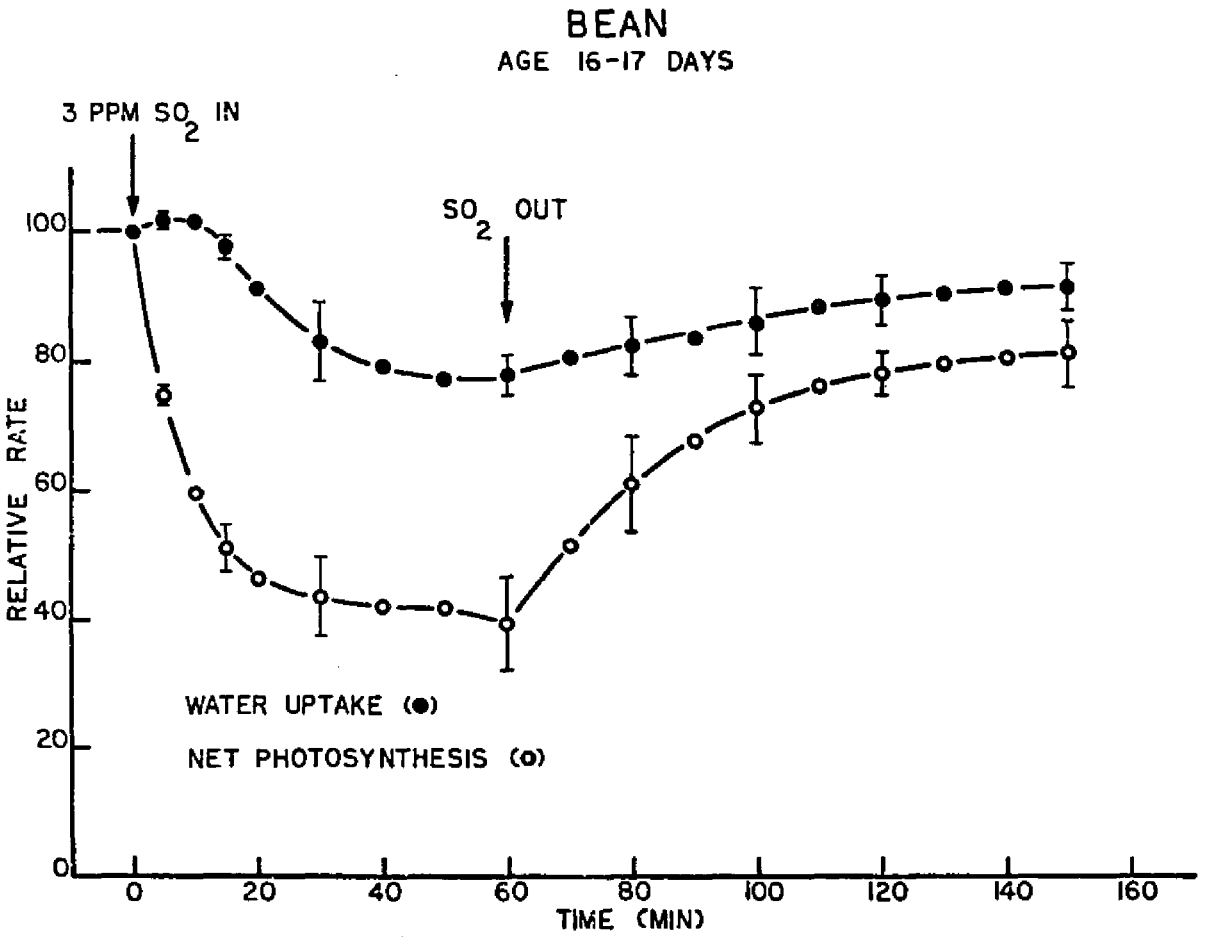
Potometric Studies

A reduction in photosynthesis could be effected by rapid closure of the stomata in response to SO_2. For present purposes, stomatal response to SO_2 was measured indirectly by determining potometrically the time-course of transpiration. In hydroponically cultured plants, water uptake has been found to be essentially an instantaneous measure of water transpired (48). If stomata close rapidly in response to SO_2, one could assume that the observed rapid decrease in the photosynthetic rate could be attributed to increased resistance to the uptake of CO_2. If so, the effect of SO_2 on photosynthesis would be secondary, at least during the initial stages of inhibition.

As shown in Figure 14 the reverse situation appeared to be the case. After introducing 3 ppm SO_2 into the leaf cuvette the photosynthetic rate decreased rapidly, approaching 75 percent of its final value within the first 15 minutes of exposure. During this same period water uptake remained relatively constant -- in fact, slightly increased.

After about 15 minutes of SO_2 treatment the rate of water up-

Figure 14--Time-course of transpiration (as a measure of the stomatal aperature) and net photosynthesis of the primary leaf of 16- to 17-day-old Pinto bean during a 1-hour exposure to 3 ppm SO_2. (100 = 1.71 $\pm$ 0.16 g dm^{-2} hr^{-1} for transpiration and 17.4 $\pm$ 2.16 mg dm^{-2} hr^{-1} for photosynthesis.) Each point is an average of 3 plants.


BEAN
AGE 16-17 DAYS
3 PPM SO2 IN
SO2 OUT
RELATIVE RATE
100
80
60
40
20
0
WATER UPTAKE (●)
NET PHOTOSYNTHESIS (o)
0
20
40
60
80
100
120
140
160
TIME (MIN)

take began to decline and reached a constant level 23 percent below the pretreatment rate by the end of the exposure period. During the same period the photosynthetic rate also reached a constant level 60 percent below the pretreatment rate. Immediately after termination of the SO_2 treatment both the water uptake rate and photosynthetic rate began to recover.

From these experiments it was concluded that the stomata did not respond fast enough to account for the large decrease in photosynthesis during the initial 15 minutes of SO_2 treatment. It appears more likely that the stomata closed in response to an increase in the partial pressure of CO_2 within the leaf caused by a reduction in the rate of photosynthesis.

It is recognized that porometry would give a more sensitive measure of the stomatal aperature. However, the measurement of photosynthetic rates concurrently with the measurement of stomatal aperature by this method would be more difficult because of the small leaf area used for sampling. In the case of forced-air or pressure porometers, photosynthetic rates would have to be corrected for the continuous change in flow rates as the stomata open and close. Also, one must correct for the continuous change in lag time from the moment the gas leaves the leaf to the time it enters the analyzer. Lag times would vary with changing flow rates. Nevertheless, forced-air porometers would aid in establishing the effect of SO_2 on stomata within the first 15 minutes of exposure.

Environmental Factors and Plant Susceptibility

Many environmental factors have controlling influences on a plant's susceptibility to a particular pollutant or combination of pollutants. Among the more significant factors are temperature, relative humidity, soil moisture content, photoperiod, light intensity, CO_2 concentration, soil fertility and wind speed (9,10,40).

For example, Mansfield and Majernik (33) have shown that after 1 hour at 1 ppm SO_2 and low relative humidity, 32 percent at 18°C, the stomatal aperature of Vicia faba L. was decreased by about one-half. At 0.5 ppm SO_2 however, and a moderate relative humidity of 42 percent at 18°C, the stomatal aperature was considerably greater than that of the controls. The conclusion drawn was that in an atmosphere of low relative humidity SO_2 suppresses stomatal opening, while in an atmosphere of high relative humidity SO_2 expedites stomatal opening.

The data of Mansfield and Majernik (33) show that differences in the degree of stomatal opening during a 12-hour exposure to 1 ppm SO_2 in Vicia faba L. were not evident until about 1 hour after SO_2 was introduced. From the short-term kinetic studies presented here one could postulate that the closure Mansfield and Majernik observed at 1 ppm SO_2 was actually due to an increase in the internal partial pressure of CO_2 resulting from an inhibition of photosynthesis. By similar reasoning, it is possible that stomatal opening at 0.5 ppm SO_2 was the result of an increased photosynthetic rate. It is indeed difficult to determine if stomata respond to SO_2 directly as

was postulated by these authors (32,33).

General Considerations of SO_2 Effects

The present studies indicate that the initial decline in net photosynthesis cannot be explained in terms of stomatal closure or by increases in either the rates of dark respiration or photorespiration. Due to the rapidity with which photosynthesis responds to SO_2, it is suggested that the photosynthetic mechanism may be directly affected by the gas.

Sulfate reduction is considered to involve the following sequence of events: $S^{(+6)}O_4^{(-2)} \rightarrow S^{(+4)}O_3^{(-2)} \rightarrow X^{(+2)} \rightarrow X^{(0)} \rightarrow S^{(-2)}$. It is believed that the intermediates designated X may actually be "protein bound". These intermediates can serve as nutritional sulfur sources for higher plants and microorganisms. For example, certain microbial mutants lacking the sulfate-reductase system were unable to grow on sulfate, but did grow on sulfite or the more reduced sulfur compounds. Sulfur oxidation, on the other hand, is not considered to be a reversal of the sulfur reduction pathway, but rather is coupled to the cytochrome system (5). Since the above studies indicate that sulfite is an intermediate in sulfur metabolism, it appears that sulfite can serve in a capacity similar to that of sulfate.

Early studies (16,30) have shown that the synthesis of sulfur-containing protein in green leaves was enhanced when the leaves were illuminated. Several authors (16,20,30) have indicated that light enhanced the reduction of sulfate in leaves, and that sulfate

reduction may be directly coupled to photosynthesis (3,30). Apparently sulfate can be reduced by photosynthesis to the thiol moiety (HS^-) with the elimination of molecular oxygen (6). Recently Cormis (11) has shown that tomato plants when exposed to $^{35}SO_2$ in the light released $H_2{}^{35}S$. These studies suggest that the rate of photosynthetic sulfate (or sulfite) reduction may be in part responsible for detoxifying a portion of the absorbed SO_2. Possibly this reduction capacity was greater in corn or in the young leaves of Pinto bean, thereby giving these leaves some degree of resistance to SO_2. A high reducing capacity could account for the constant rate of photosynthesis observed in corn after a 30-minute exposure to 3 ppm SO_2 and the greater recovery levels in photosynthesis in the young leaves of bean.

Figure 13 shows that the photosynthetic rates in both bean and corn reached constant values 2 hours after exposure to 1 ppm SO_2. At this level of SO_2, the rate of photosynthetic sulfate or sulfite reduction may have kept sulfite or sulfate levels below toxic threshold limits during the 4-hour exposure period, thus allowing for normal photosynthetic rates to resume once SO_2 was removed. One may conclude from the data presented in Figure 12 that in bean the rate of SO_2 absorption, at a concentration level of 3 ppm, exceeded the sulfate and sulfite rate of reduction thus leading to degenerative changes in the photosynthetic apparatus. Corn, by similar reasoning, was able to withstand this condition.

A possible contributing factor leading to SO_2 resistance may be correlated with the type of carbon fixation present in the plant.

Corn, sorghum, and Johnson grass are reported to be SO_2 resistant (7). These particular plants have low compensation points (17), thus indicating a low level of photorespiration (28). The fact that these species exhibit a reduced level of photorespiration indicates that the enzymes involved in carbon reduction have a very high affinity for CO_2. Perhaps the affinity of enzymes (or even the number of active enzymes) essential for sulfite and sulfate reduction is also high in these plants. It is of interest to note that according to Bandurski (5) the electron donor for sulfate and sulfite reduction may not be reduced pyridine nucleotide which is utilized directly in CO_2 reduction but a "photosynthetically generated reductant (such as ferredoxin)". It is possible that in plants having a low level of photorespiration the enzymes involved in sulfite and sulfate reduction compete more favorably for reduced ferredoxin than those enzymes having a similar function in plants with a high level of photorespiration. In the light of Cormis' work (11), experiments designed to determine the rates of SO_2 reduction in illuminated leaves of plants of varying sensitivity would aid in the understanding of the mechanism of SO_2-resistance in plants.

One fact evident in all of the present studies was the rapidity (within 2 minutes) with which photosynthesis responded to SO_2. Therefore, any hypothesis concerning the mechanism of inhibition of photosynthesis by SO_2 must take into account the rapid kinetics of this response.

The following hypothesis for the rapid inhibition of photosynthesis in the presence of SO_2 is suggested. We assume that the

pH of the cytoplasm is approximately 6.8 to 7.0, typical of many plant species (36). The data of Vass and Ingram (cited by Gilbert [23]) show that in an aqueous solution of SO_2 at pH 7.0, 95 percent of the ions are in the form of the sulfite ion SO_3^{-2} (as opposed to the bisulfite ion HSO_3^{-1}). An aqueous solution containing 10 mM sodium sulfite has been shown to inhibit photophosphorylation by 75 percent in whole spinach chloroplast extracts (1). Further, the concentration of sulfite required to cause a 50 percent inhibition of $^{14}CO_2$-incorporation in illuminated chloroplasts was lower than that required to cause a 50 percent inhibition of photophosphorylation (1). As stated above, the solution product of SO_2 is the sulfite ion which may be oxidized to the sulfate ion by the cell. Therefore, during SO_2 treatment the cell probably contains both sulfite and sulfate ions. Asada (1,2) has shown that the same molar concentrations of either sulfite or sulfate can reduce photophosphorylation almost equally. Ryrie and Jagendorf (37) have demonstrated that a 10 mM potassium sulfate solution plus 40 to 180 seconds of illumination reduced photophosphorylation in chloroplasts by almost 50 percent. Inhibition of photophosphorylation occurred rapidly with a half-time of about 10 seconds. Accepting Saunders' calculations (38) that 100 µg SO_2 per cubic meter of air (approximately 0.04 ppm v/v at 25°C [19, p. 122]) yields at equilibrium with water a solution containing 35 ppm SO_2, then 3 ppm SO_2 in air would yield approximately 2600 ppm SO_2 in an aqueous solution. Assuming that the SO_2 is converted primarily to sulfite and sulfate and that these ions

are uniformly distributed throughout the cytoplasm, then the molar concentration at equilibrium will be about 40 mM, well above the range of sulfite and sulfate concentration found to be 50 percent inhibitory to photophosphorylation.

Ryrie and Jagendorf (37) and Asada (2) indicated that sulfate ions inhibited both cyclic and noncyclic photophosphorylation. However, the electron flow from water to the electron acceptor ferricyanide was not impaired. Thus they concluded sulfate inhibition resulted in a partial uncoupling of the phosphorylation reactions. Specifically, sulfate altered photophosphorylation by interacting with CF_1 (chloroplast-coupling factor 1) which was responsible for catalyzing the terminal phosphorylation reactions (37). Ryrie and Jagendorf (37) suggested that a large part of the CF_1 in the chloroplast was irreversibly damaged by sulfate. These facts are significant in view of the present experiments in which a portion of the photosynthetic apparatus in bean may have been permanently damaged (recovery to the pretreatment rate was not complete 2 hours after SO_2 exposure) when exposed to 3 and 5 ppm SO_2. In relation to the present studies, sulfite or sulfate inhibition of photophosphorylation was rapid enough to account for the rapid decrease in photosynthesis.

If one of the effects of sulfite or sulfate ions is the inhibition of photophosphorylation, then photosynthesis should be substantially reduced in all plants regardless of their age or sensitivity to SO_2 during the actual exposure period to SO_2. Figures 11 and 12 showed that for either the sensitive bean or the resis-

tant corn, photosynthesis was greatly reduced.

Some highly preliminary investigations using the electron microscope have shown that after a 1-hour treatment with 3 ppm SO_2 the thylakoids appeared to enlarge and separate leaving "holes" in the grana. This was the first morphological change to be detected in the chloroplast after SO_2 exposure (personal communication -- Mr. C. Krause, United States Department of Agriculture, Delaware, Ohio). This finding substantiates the speculation that the photosynthetic process was directly affected by SO_2 and may involve the inhibition of an enzyme(s) essential for photosynthesis. A more detailed cytological study should be undertaken to further develop these observations.

Several studies have shown (4,37) that either phosphate or arsenate can compete with sulfate, thus protecting photophosphorylation against sulfate inhibition. Perhaps the phosphate levels of leaves or soil can be correlated with plant resistance. Since low soil fertility may increase plant sensitivity to air pollutants (40), high phosphate levels may give some added protection to plants. Investigations involving soil nutrition and plant sensitivity are needed in light of the competitive nature of phosphate with sulfate.

Another line of investigation suggested by the present studies would be a study of the change in pH of the cells during exposure. Sulfur dioxide toxicity and a pH effect may be difficult to separate since the pH of an aqueous solution of SO_2 is largely determined by the amount of SO_2 absorbed (39). (The pH of a cell and its buffer capacity could determine to a large extent the distribution of

toxic compounds in solution [23]). It would be of interest to determine the length of time a plant cell maintains a give pH during SO_2 exposure before its buffer capacity is exceeded. It is possible that the lowering of the cell pH is rapid enough to be correlated with the sharp decrease in photosynthesis that is observed when a leaf is exposed to SO_2. High SO_2 levels may rapidly cause the buffer capacity to be exceeded in some cells resulting in "permanent" injury to membranes, causing chlorosis (19) or permanent loss in the photosynthetic capacity in these areas of the leaf. If the buffer capacity in some cells were exceeded, this could account for the "permanent" reduction in photosynthesis observed when leaves were exposed to 3 or 5 ppm SO_2. It seems unlikely, however, that at low levels of SO_2 the immediate decrease in photosynthesis would be due to a pH change.

CHAPTER III

SUMMARY

The effects of 1-hour exposures to 1, 3 and 5 ppm SO_2 on photosynthesis in the primary leaf of Pinto bean was studied as a function of leaf age. In the present studies 13-, 17- and 21-day-old primary leaves (representing young, middle-aged and old leaves respectively) all showed substantial and generally proportionately equal decreases in net photosynthesis at any given SO_2 concentration. Thus, inhibition of the photosynthetic process appears to be independent of leaf age in Pinto bean, a species reportedly sensitive to SO_2. Although the time-course of inhibition was notably uniform between individual test plants, greater variability was noted during the recovery phase. It is suggested that variations in susceptibility to SO_2 depend principally on differences in genetically determined capacities for restoration of tissue or organelles affected by SO_2. Since in 17-day-old leaves the average relative rates of photosynthesis following a period of SO_2 inhibition were less than those of 13- and 21-day-old leaves, it was concluded that the capabilities for repair of tissue injured by SO_2 may be greater in younger and older leaves than in leaves of intermediate age.

For comparative purposes similar inhibition time-curves were determined for corn, a species reported to be tolerant to SO_2.

Exposure of the third leaf of 17- to 21-day-old corn plants to 1 ppm SO_2 resulted in inhibition and recovery kinetics almost identical to the kinetics of bean similarly treated with 1 ppm SO_2. However, 30 minutes after the introduction of 3 ppm SO_2, photosynthesis in corn had reached a constant level 50 percent below the pretreatment rate, while photosynthesis in bean still showed a steady decline to about 25 percent of the pretreatment rate by the end of the exposure period. It was concluded that corn has some means of protecting the photosynthetic mechanism during exposure to SO_2, while in bean this mechanism was apparently stressed beyond threshold limits.

In both bean and corn a 4-hour exposure to 1 ppm SO_2 resulted in a 15 to 20 percent reduction in photosynthesis during the first hour which was maintained during the subsequent 3 hours. Since photosynthesis in both plants recovered to the pretreatment rate within 1 hour after the exposure period, it was inferred that the repair capacity of the mechanism resulting in recovery had not been exceeded at this concentration level in either species.

Potometric studies indicated that water uptake (as a measure of transpiration and the degree of stomatal opening) began to decrease 15 minutes after the introduction of 3 ppm SO_2, a period during which the rate of photosynthesis fell close (within 25 percent) to its final value. Hence, it appears unlikely that the kinetics of inhibition during the early phase of SO_2 exposure can be ascribed to stomatal closure. More likely the stomata close

in response to a higher CO_2 partial pressure resulting from a reduced rate of photosynthesis. Increases in dark respiration or photorespiration during exposure to SO_2 were tentatively eliminated as causative factors leading to the reduction in the apparent net photosynthetic rate.

It was noted that many (but not all) areas of the leaf visibly damaged by SO_2 (collapsed areas produced by a 1-hour exposure to 5 ppm SO_2) recovered their normal appearance several hours after treatment. Thus at both physiological and macroscopic levels, evidence was obtained that SO_2 damage is reversible if the system is not stressed beyond the threshold limit.

In plants which appear tolerant to low levels of SO_2, it is suggested that SO_2 resistance may be correlated, in part, with the greater ability of these plants to detoxify the absorbed SO_2 to the less toxic sulfate form and to reduce the sulfate and sulfite ions (solution product of SO_2) to organic sulfhydryls or to hydrogen sulfide which is subsequently released to the atmosphere.

On the basis of present investigations it is concluded that SO_2 has a direct inhibitory effect on the photosynthetic mechanism. It is postulated that this inhibition results from the accumulation of sulfite and sulfate ions in concentrations that severely and rapidly restrict photophosphorylation.

APPENDIX A

OMNITAB (OCT 15, 1970, STANDARD CURVE POLYNOMIAL FIT, J. W. SIJ)

```
READ THE FOLLOWING DATA INTO COLUMNS 2,3
 284.0      0.0
 291.0      3.8
 297.6      9.0
 304.5     14.1
 317.5     23.5
 337.5     38.4
 351.0     48.1
 371.0     65.1
 378.0     69.5
 385.0     74.7
 399.6     81.3
 406.0     86.0
 420.0     95.0
ADD 1.0 TO 0.0 AND STORE IN COL 4
POLYFIT Y IN 2, WTS IN 4, X IN 3, DEG 2, STORE COEF IN 5, RES IN 6
POLYFIT Y IN 2, WTS IN 4, X IN 3, DEG 3, STORE COEF IN 7, RES IN 8
POLYFIT Y IN 2, WTS IN 4, X IN 3, DEG 4, STORE COEF IN 9, RES IN 10
SUBTRACT COL 6 FROM COL 2 AND STORE IN COL 11
SUBTRACT COL 8 FROM COL 2 AND STORE IN COL 12
SUBTRACT COL 10 FROM COL 2 AND STORE IN COL 13
TITLEY COMPUTED CO2 CONC. USING 3 POLY FITS
TITLEX   ACTUAL CO2 CONCENTRATION
PLOT COL 11,12,13 VS COL 2 ABS FROM 284.0 TO 420.0
TITLEY COMPUTED CONC. AND ACTUAL CONC.
TITLEX                METER READING
PLOT COL 11,2 VS COL 3 ABS FROM 0.0 TO 100.0
PLOT COL 12,2 VS COL 3 ABS FROM 0.0 TO 100.0
PLOT COL 13,2 VS COL 3 ABS FROM 0.0 TO 100.0
TITLEY RESIDUALS  DEGREE 2
PLOT COL 6 VS COL 3 ABS FROM 0.0 TO 100.0
TITLEY RESIDUALS  DEGREE 3
PLOT COL 8 VS COL 3 ABS FROM 0.0 TO 100.0
TITLEY RESIDUALS  DEGREE 4
PLOT COL 10 VS COL 3 ABS FROM 0.0 TO 100.0
FIXED WITH 5 DIGITS AFTER THE DECIMAL PT
HEAD COL 2 /ACT CO2 CONC
HEAD COL 3 /SCALE READING
```

```
HEAD COL 11 /CMP CO2 DEG2
HEAD COL 12 /CMP CO2 DEG3
HEAD COL 13 /CMP CO2 DEG4
HEAD COL 5 /COEF DEG2
HEAD COL 7 /COEF DEG3
HEAD COL 9 /COEF DEG4
HEAD COL 6 /RESIDLS DEG2
HEAD COL 8 /RESIDLS DEG3
HEAD COL 10/RESIDLS DEG4
PRINT COL 3,2,11,12,13,5,7,9
PRINT COL 3,2,6,8,10
STOP
END
```

APPENDIX B

```
      DIMENSION X(100), Y(100)
      A=2.8431445E 02
      B=1.5608368E 00
      C=-7.2834454E-03
      D=6.3205342E-05
C     A,B,C,D, ARE THE COEF FROM A THIRD DEGREE POLYNOMIAL FIT
      X(1)=0.0
      DO 1 J=1,20
    1 X(J+1) = X(J) + 5.0
      DO 2 K=1,21
    2 Y(K)=A + B*(X(K)) + C*(X(K)**2) + D*(X(K)**3)
      WRITE(6,10)
   10 FORMAT (1H1,'METER READING ',10X,'CO2 CONC (PPM)')
      WIRTE (6,11) (X(J),Y(J),J=1,21)
   11 FORMAT (1HO,4X,F10.3,14X,F10.3)
      STOP
      END
```

APPENDIX C

```
      DIMENSION X(500),Y(500),CO2DIF(500),AMGCO2(500),AB(80),O(80),
     *H(80),I(1428),RELRTE(500),TIME(100),T(500)
C H=HEADING OF PLOT, O=ORDINATE LABEL, AB=ABSCISSA LABEL
      READ (5,11) (O(J),J=1,20), (AB(J),J=1,20)
C N=NUMBER OF DATA POINTS, SPAN=CONC. OF UPSPAN GAS USED (IN PPM),
C AREA=AREA OF LEAF IN CM2, SO2=SULFUR DIOXIDE CONC. USED IN THE
C EXPERIMENT IN PPM.
C SPEC=NAME AND NUMBER OF EXPERIMENT, DATE=DATE EXPT. WAS RUN,
C TREAT=AGE OF PLANT USED ALONG WITH CONC OF SO2 AND THE DURATION
C OF THE SO2 TREATMENT
  101 READ(5,10,END=100)N,SPAN, AREA, SO2,(H(J),J=1,15)
C DATA ARE READ IN AT 5 MIN INTERVALS.
C EACH POINT REPRESENTS AN AVERAGE CO2 CONC. OVER A ONE MINUTE IN-
C TERVAL, I.E. PLUS OR MINUS 30 SECONDS
      READ(5,12) (X(K),K=1,N)
C A,B,C,D=COEFFICIENTS OF THE 3RD DEGREE POLYNOMIAL FIT FROM OMNITAB.
C CHAR=THE SYMBOL USED WHEN PLOTTING THE DATA
      DATA A,B,C,D,CHAR/2.8431445E+02,1.5608368E+00,-7.2834454E-03,
     *6.3205342E-05,'   .'/
      TIME(1)=0.0
      DO1 K=1,N
      IF(K.EQ.1.OR.K.GT.13) GOTO 30
C DURING SO2 FUMIGATION, THE CONC. OF CO2 IN THE SO2 TANK MAY NOT
C EQUAL THAT OF THE SPAN GAS, THEREFORE A CORRECTION MUST BE USED TO
C TAKE THIS CO2 DIFFERENCE INTO ACCOUNT
C 53.2 EQUAIS THE CONC. OF SO2 IN PPM IN THE SO2 SUPPLY TANK.
      RATIO=SO2/53.2
      DIFF=1.-RATIO
C 427.75 EQUALS THE CO2 CONC. IN THE SO2 SUPPLY TANK.
      SPNDIF=(SPAN*DIFF = 427.75*RATIO) - SPAN
C IN THE WORKING RANGE OF THE STANDARD CURVE, THERE ARE APPROXI-
C MATELY 1.44 PPM CO2 INCREASE PER UNIT METER READING (ONE UNIT=
C ONE 100TH OF FULL SCALE).
      UNITS=SPNDIF/1.44
C WATER VAPOR ACCOUNTS FOR AN INCREASE OF 4.75 CHART UNITS.
      TOTUN=UNITS + 4.75
      GOTO 31
   30 TOTUN=4.75
C T IS THE TRUE OR CORRECTED CO2 UPTAKE
   31 T(K)=X(K)-TOTUN
      TIME(K+1)=TIME(K)+5.0
```

```
C EQUATION FROM OMNITAB TO APPROXIMATE STANDARD CURVE OF CO2
C CONC. VS METER READING.
      Y(K)=A + B*(T(K)) + C*(T(K)**2) + D*(T(K)**3)
      CO2DIF(K)=SPAN-Y(K)
C CALCULATION OF MILLIGRAMS OF CO2 UPTAKE PER SQUARE DECIMETER
C PER HOUR.
C ASSUME STANDARD CO2 TANK CALIBRATED AT 1 ATMOSPHERE AND 25 DEG
C C. LET V=NRT/P, WHERE N=P=1., R=0.0820549 L-ATM DEG-1K MOLE-1.
C V=298XR=24452.36 ML.  ONE MOLE CO2=44010. MG.  SO, ML/MG =
C 0.555609 ML PER MG NORMALIZED TO AN AMBIENT CO2 CONCENTRATION OF
C 420. PPM.
      AMBCO2(K)=(420.*CO2DIF(K)*6.)/(AREA*.555609*SPAN)
C RELATIVE RATE CALCULATED--T RATE/BASE RATE I.E. PRETREATMENT RATE
    1 RELRTE(K)=(AMGCO2(K)/AMGCO2(1))*100.
      WRITE(6,20) (H(J),J=1,15)
      WRITE(6,21)
      WRITE(6,22)(TIME(K),X(K),T(K),Y(K),AMGCO2(K),RELRTE(K),K=1,N)
C PLOT RELATIVE RATE VS TIME DURING AND AFTER A 1 HOUR SO2 TREATMENT.
      CALL PLOTA (I,0.0,TIME(N+1),-10.,110.,1)
      CALL PLOTB (TIME, RELRTE,CHAR,N)
      CALL PLOTC (H,15,0,12,AB,20)
      WRITE(6,23) AMGCO2(1)
      GOTO 101
   10 FORMAT(I5,3F5.1,15A4)
   11 FORMAT(20A4)
   12 FORMAT(16F5.2)
   20 FORMAT(1H1,30X,15A4)
   21 FORMAT(1HO,'TIME (MIN) ',5X,'METER READING',5X,'CORRECTED
     *READING',5X,'PPM CO2',5X,'CO2 UPTAKE(MG DM-2 HR-1)',5X,
     *'RELATIVE RATE'/)
   22 FORMAT(1H,F10.1,5X,F10.2,10X,F10.2,8X,F10.2,10X,F10.2,13X,
     *F10.2)
   23 FORMAT(1HO,'PRETREATMENT PHOTOSYNTHETIC CO2 UPTAKE RATE=',
     *F10.5,'MG CO2 PER DM2 PER HOUR = 100 ON RELATIVE RATE BASIS')
  100 STOP
      END
```

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