The Influence of Soil Moisture on Macroscopic Sulfur Dioxide Injury to Pinto Bean Foliage

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ABSTRACT

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The influence of soil moisture stress on sulfur dioxide (SO_2) injury to pinto bean foliage was investigated in relation to stomatal conductance rate, soil moisture content, and plant water potential. Pinto bean plants were grown at four soil water potentials ($-1/3$, -1, -3, and -5 atm) and exposed to 5,720 $\mu g/m^3$ (2.2 ppm) SO_2 for 3 hr. Macroscopic injury was severe on plants grown at $-1/3$ and -1 atm soil water potential and negligible on plants grown at -3 and -5 atm water potential. Injury was highly correlated with percentage of soil moisture, and both injury and soil moisture were highly correlated with stomatal conductance rate and water potential of the plants. The duration of soil moisture stress (1, 2, or 3 days) did not affect the amount of macroscopic injury induced by SO_2, the stomatal conductance rate, or plant water potential. Stomatal conductance rates of plants grown at $-1/3$ and -1 atm soil water potential decreased when the plants were exposed to SO_2, while those of plants grown at -3 and -5 atm soil water potential were not affected by exposure to SO_2.

Changes in soil moisture have been reported to influence plant sensitivity to sulfur dioxide (SO_2); however, most of the experiments have been very general in nature. Alfalfa (*Medicago sativa* L.) and buckwheat (*Fagopyrum esculentum* Moench) plants grown under optimal water conditions were generally more sensitive to SO_2 than those grown under water stress, even when adequate soil moisture was available to both groups at the time of exposure (19). Katz (9) reported that "turgid" plants grown in soil containing 12.8% moisture sorbed more SO_2 and were more sensitive to SO_2 than wilted plants grown in soil with 6.2% moisture. Temporary dehydration of leaf tissue in broadbean (*Vicia faba* L.) and barley (*Hordeum vulgare* L.) induced by drought stress raised tolerance to SO_2 and decreased sorption of SO_2 compared with plants grown at field capacity (14). Schramel (18) reported that chronic SO_2 gassing of six drought-stricken crops (including *V. faba* 'Hangdown White') caused stomata to widen but had no effect on stomatal resistance measured with a pressure porometer. Dehydrated plants grown under drought stress showed increased stomatal resistance, which was correlated with greater tolerance to SO_2.

In most of these studies, soil moisture content was not critically controlled or accurately measured. Interrelationships among SO_2 injury, soil moisture, and plant water potential have not all been investigated at once. We attempted to evaluate the influence of soil moisture regimes on the sensitivity of pinto bean plants (*Phaseolus vulgaris* L. 'Pinto 111') to SO_2, as related to plant water potential, stomatal conductance rate, soil moisture content, and plant growth. Data were collected from December 1978 through May 1979.

MATERIALS AND METHODS

Establishing soil water potential. Soil water potential can be maintained at desired levels by varying concentrations of polyethylene glycol (PEG) in the root zone (10,16,23,26–29). However, plants grown hydroponically in this solution may absorb

some PEG (7,11), altering physiologic processes, which in turn may influence plant responses to other stresses such as exposure to SO_2. This problem may be minimized by separating the soil and roots from the PEG with a cellulose dialysis membrane that excludes compounds with molecular weights (mol wt) greater than 12,000 and using a 20,000 mol wt PEG (23). The technique we used is a modification of that presented by Tingey and Stockwell (23).

Pinto bean seeds were germinated in trays of vermiculite in a "preexposure" growth chamber maintained at 27 ± 0.5 C and $75 \pm 2\%$ relative humidity (RH), with a 12-hr photoperiod of 33 klux beginning at 0600 hours. Six days after seeding (2 days after cotyledon emergence), 32 seedlings of uniform development were transplanted into steam-aerated Hagerstown silt loam that had been strained through a 7-mm mesh screen and drenched with distilled, deionized water. The soil was contained in 20×4.9 cm tubes of cellulose dialysis membrane that excluded compounds with molecular weight greater than 12,000, thereby preventing phytotoxicity from the 20,000 mol wt PEG. The tubes were sealed at one end.

Each of four sets of eight plants, with roots and soil enclosed in the cellulose membrane, was placed in a 10-L vessel containing a solution of distilled, deionized water and PEG 20,000. The tubes were held upright by a piece of foam insulation 17 mm thick. The ratio of PEG to water in the four vessels was varied to maintain soil water potentials (26) at $-1/3$, -1, -3, or -5 atm (1 atm = 1.013 bars). Water used by the plants was replaced to maintain a maximum amount of solution in each vessel.

Excessive nutrient concentrations in the soil or in the PEG solution reportedly lead to rapid deterioration of dialysis membranes (27). Plant growth and visible response to SO_2 were similar for plants grown in unamended topsoil placed in either a mixture of distilled, deionized water and PEG or in Hoagland's solution (5) and PEG mixture (J. A. Davids, *unpublished*). Soil was analyzed and found to be adequate for normal plant growth without a nutrient supplement; therefore, to prolong the usefulness of the dialysis membrane, all plants were grown in unamended topsoil surrounded by a water and PEG solution.

Characterization of plant material. Seedlings were transplanted 6 days after sowing; four sets of eight plants were then grown for 4 days in membrane-encased soil surrounded by the PEG solution (preliminary experiments revealed that plants reached maximum sensitivity to SO_2 10 days after seeding). At this time, the biomass of

the roots and shoots was determined for each of the four soil water potentials. Subsets of six plants were removed from each solution, the dialysis membranes were removed, and the roots were washed thoroughly. Fresh weight of roots, shoots, and leaves was recorded. Plant parts were placed in an oven at 100 C until they reached a constant weight, when dry weight was determined. Leaf area was determined with a portable area meter (Lambda Instrument Corp., Lincoln, NE 68501). This experiment was replicated three times and was separate from the following experiments. Data were analyzed by analysis of variance, and means were separated ($P = 0.05$) with Duncan's new multiple range test (22).

SO₂ exposure system. The SO_2 dosage at which foliar injury developed on plants growing under the lowest soil moisture stress was found to be 5,720 $\mu g/m^3$ (2.2 ppm). Ten days after seeding, plants growing at the four levels of soil water potential were exposed simultaneously to 2.2 ppm SO_2 for 3 hr at 27 C, 75% RH, and 45 klux light intensity within a controlled-environment exposure chamber (Model M-2, Environmental Growth Chambers, Chagrin Falls, OH 44022). All exposures began at 1000 hours. The SO_2 was delivered from a tank of 100% SO_2 through stainless steel lines to the exposure chamber, where it mixed with incoming charcoal-filtered air before reaching the plants. Sulfur dioxide concentrations in the chamber were monitored with a pulsed fluorescent SO_2 analyzer (Thermo Electron Corp., Hopkinton, MA 01748) calibrated periodically with certified permeation tubes (15) that were reweighed in our laboratory.

After exposure, the plants were returned to the preexposure chamber and maintained in the same environmental conditions in which they were grown. Twenty-four hours after exposure, the percentage of visibly injured tissue of each unifoliolate leaf was estimated to the nearest 10%; in addition, values of 1, 5, 95, and 99% were included at the extremes.

Duration of soil moisture stress. Preliminary studies revealed that each desired soil water potential was attained 24 hr after the encased soil was placed in the PEG solution. That is, plants grown for 4 days in the solution were subjected to at least 3 days of the desired water potential. Plants to be subjected to only 1 or 2 days of stress were maintained in the tubes in distilled, deionized water for 2 days or 1 day, respectively, before being transferred to the PEG solution.

Before exposure to SO_2, two plants were selected randomly from each treatment. Stomatal conductance was measured at 0940 hours on the abaxial surface of one unifoliolate leaf per plant with an electrical diffusive resistance porometer (Lambda Instrument Corp.) periodically calibrated with a calibration plate of known resistance.

Two plants were selected randomly from each treatment and excised with a razor blade 2 cm below the cotyledons. Plant water potential was measured with a pressure bomb (25) before exposure. The cellulose membrane tubes of the sacrificed plants remained in place until after the vessels were removed from the exposure chamber. The lowest 10 cm of the tube was then excised, and about 40 g of rootfree soil was removed. The soil was dried at 105 C and percentage of soil moisture was determined on a dry-weight basis.

The remaining six plants at each soil moisture potential were exposed to SO_2 as described above after 1, 2, or 3 days of soil moisture stress, and the percentage of SO_2 injury on the 12 unifoliolate leaves of the six plants in each treatment was estimated. Correlation coefficients were calculated among percentage of soil moisture, percentage of macroscopic injury, stomatal conductance rate, plant water potential, and soil water potential.

Plants were always stressed and treated in groups containing all four soil water potentials (−1/3, −1, −3, and −5 atm). Four replicates of each treatment were exposed to SO_2 at each of the three stress times (1, 2, and 3 days). The design was a nested factorial (treatments within days of stress) using a total of 384 plants. Transformation of percentage of soil moisture and percentage of macroscopic injury data was not necessary; the original data had more suitable variance properties for analysis. Data were analyzed by analysis of variance, and means were separated ($P = 0.05$) with Duncan's new multiple range test (22).

Stomatal conductance. Sets of eight plants were grown at the four soil moisture levels for 3 days (4 days in PEG) and exposed to SO_2 as described above from 1000 to 1300 hours. Stomatal conductance was measured on one unifoliolate leaf per treatment at approximately 30-min intervals before (0930 and 0955 hours), during (1030, 1100, 1130, 1200, and 1230 hours), and after (1255, 1330, 1400, 1430, and 1500 hours) exposure to SO_2. Percentage SO_2 injury was estimated as described above on both unifoliolate leaves of each plant. This experiment was replicated three times, using a total of 96 plants (eight plants, four treatment levels, and three replicates). Stomatal conductance was also measured on one leaf of 64 unexposed plants. Data were analyzed by analysis of variance, and means were separated ($P = 0.05$) with Duncan's new multiple range test (22).

RESULTS

Characterization of plant material. Leaves from pinto bean plants grown at −1/3 and −1 atm treatments appeared to develop normally and were significantly larger than those grown at −3 and −5 atm treatments. Mean leaf area was not significantly different between the −1/3 and −1 atm treatments or between the −3 and −5 atm treatments (Fig. 1). Fresh and dry weights of roots, shoots, and leaves were greatest on plants grown at −1/3 atm of soil water potential and were generally lower at the −1 and −3 atm treatments. Plant part weights from the −5 atm treatment were generally not significantly different from those from the −3 atm treatment.

Foliar response to SO₂. The length of treatment (1, 2, or 3 days of moisture stress) had no significant influence on percentage of macroscopic SO_2 injury, percentage of soil moisture, stomatal conductance rate, or plant water potential values. Therefore, data were pooled and an overall mean value for each variable was calculated (Table 1). The degree of tan, interveinal necrosis caused by SO_2, percentage of soil moisture, and rate of stomatal conductance were greatest at −1/3 atm and lessened with decreasing soil water potential. However, mean values from the −3 and −5 atm treatments did not differ significantly from each other (Table 1). Plant water potential values also became more negative with decreasing soil water potential; however, only the means at
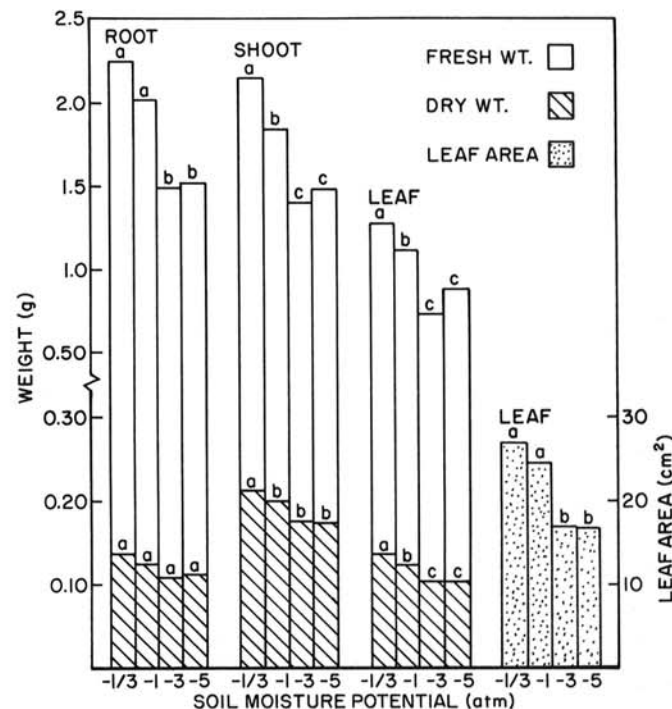

Fig. 1 Fresh weight, dry weight, and leaf area of pinto bean plants grown for 3 days at soil water potentials of −1/3, −1, −3, and −5 atm. Columns within a group topped by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test. The letters of significance should not be compared among plant parts or between fresh and dry weight.

−1/3 and −5 atm were statistically different.

Plants grown in soil with a moisture content above 23% (independent of days of stress) were much more susceptible to SO_2 than those grown in soil with moisture content below 23% (Fig. 2). All variables measured in this phase of the study were significantly correlated (Table 2).

Stomatal conductance. Before exposure to SO_2, at 0930 and 0955 hours, stomatal conductance rates in the preexposure chamber were about 0.4–0.5 cm/sec for plants growing under −1/3 and −1 atm soil moisture stress and 0.1 cm/sec for plants in the −3 and −5 atm treatments (Fig. 3).

Conductance rates of leaves on plants grown in the −1/3 atm solution at first rose sharply when plants were placed in the exposure chamber containing SO_2. As exposure progressed, conductance rates fell in the −1/3 and −1 atm treatments but were largely unaffected in the −3 and −5 atm treatments.

Severe water-soaking caused by SO_2 was noted at 1130 hours on the leaves of plants in the −1/3 atm treatment, and slightly less water-soaking was observed at this time on plant leaves from the −1 atm treatment. Water-soaking corresponded to a large decrease in stomatal conductance between 1100 and 1130 hours at those two soil moisture regimes (Fig. 3).

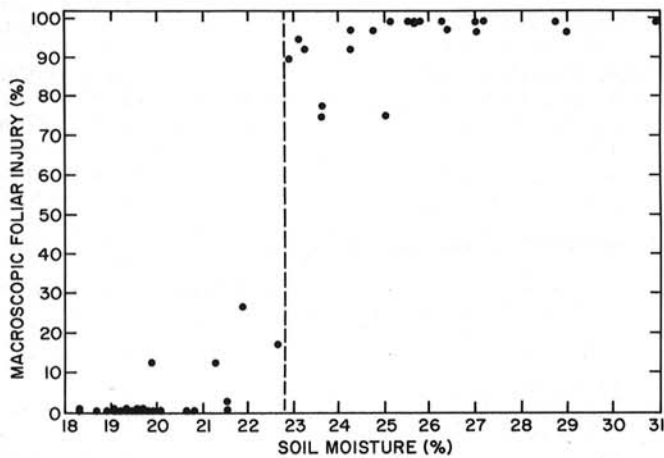The stomatal conductance rate of plants in the −1/3 and −1 atm

treatments continued to decrease during exposure until 1400 hours, when the leaf tissue was too water-soaked to allow measurements to be taken. After exposure, the stomatal conductance rate of leaves from plants grown in the −1 atm treatment rose sharply upon return to the preexposure chamber, then fell. The stomatal conductance of plants exposed to SO_2 under −3 and −5 atm soil moisture stress was not affected by any treatment. Foliar injury induced by SO_2 averaged 96, 83, 0, and 0%, respectively, for the −1/3, −1, −3, and −5 atm treatments.

When control plants were moved from the preexposure chamber to the exposure chamber without SO_2, the stomatal conductance rate of plants grown under −1/3 and −1 atm treatments increased almost sixfold to a peak of more than 2.3 cm/sec (Fig. 4). The conductance rate of plants in the −3 and −5 atm treatments did not increase. The conductance rate of unexposed control plants decreased when the plants were returned to the preexposure growth chamber, while that of plants exposed to SO_2 remained at a minimum upon return to the preexposure chamber.

DISCUSSION

The range of plant water potentials from −1/3 to −3 atm is not generally considered extremely damaging to bean plants in the field (6,21) but induced stunting in our experiment. Water was sufficient for plant viability in all treatments, and plants did not visibly wilt at any time. Apparently, processes other than those associated with visible wilting were responsible for the growth reductions observed. Effects on cell expansion, one of the processes most sensitive to water stress in plants (6), may have contributed to the reduced growth rate.

The relationship between soil moisture content and percentage of macroscopic injury resembled the general trends reported by others (9,14,19). However, large changes in macroscopic SO_2 injury corresponding to relatively small differences in soil moisture content have not been reported previously and illustrate that soil moisture is critical to pinto bean plant response to SO_2 in this system, especially for soil moisture contents near 23%.

Fig. 2. Percentage of macroscopic injury induced by SO_2 on unifoliolate leaves of pinto bean plants grown under varying percentages of soil moisture.
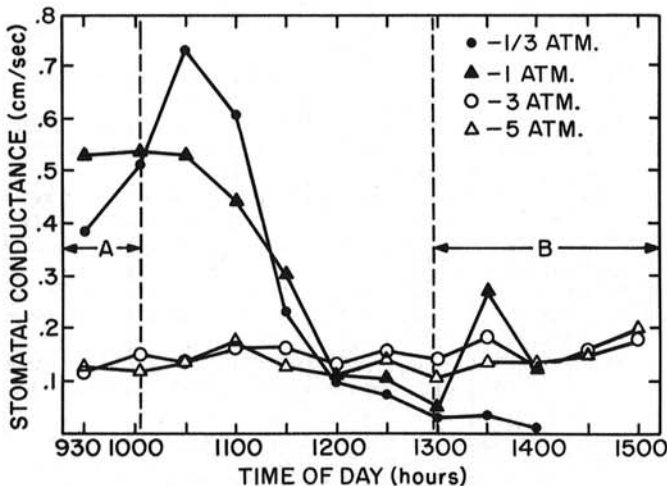

Fig. 3. Stomatal conductance rates of unifoliolate leaves of pinto bean plants grown under varying soil water potential before (A), during, and after (B) exposure to 2.2 ppm SO_2 at 27 C, 75% relative humidity (RH), and 45 klux light intensity. Before and after exposure, plants were grown at 27 C, 75% RH, and 33 klux light intensity. Each point is the average of three replicates.

TABLE 1. Mean percentage SO_2 injury, percentage soil moisture, stomatal conductance rate, and plant water potential of pinto bean plants grown at −1/3, −1, −3, and −5 atm of soil water potential

Soil water potential (atm)	SO_2 injury[x,y] (%)	Soil moisture[y,z] (%)	Conductance rate[y,z] (cm/sec)	Plant water potential[y,z] (atm)
−1/3	97.8 a	27.0 a	0.224 a	−1.05 a
−1	77.0 b	23.8 b	0.196 b	−1.41 ab
−3	2.3 c	20.1 c	0.107 c	−2.72 ab
−5	0.0 c	19.4 c	0.091 c	−3.41 b

[x]Each mean is the average of 144 values (12 measurements, three treatment lengths, four replicates).
[y]Values in a column followed by different letters are significantly different ($P = 0.05$).
[z]Each mean is the average of 24 values (two measurements, three treatment lengths, four replicates).

TABLE 2. Correlation coefficients among soil water potential, percentage of soil moisture, percentage of macroscopic injury, stomatal conductance rate, and plant water potential for pinto bean plants exposed to SO_2[a]

Parameter	Soil water potential	Percentage SO_2 injury	Percentage soil moisture	Conductance rate
Percentage SO_2 injury	0.889			
Percentage soil moisture	0.865	0.905		
Conductance rate	0.801	0.838	0.820	
Plant water potential	−0.690	−0.639	−0.591	−0.574

[a]Each correlation coefficient is significant ($P = 0.05$).

Plants grown at $-1/3$ and -1 atm soil moisture stress had much higher stomatal conductance rates (and SO_2 uptake) than those grown at -3 and -5 atm, corresponding to the amount of injury induced by SO_2 at each treatment. These results agree with Schramel's (18) general report that decreased SO_2 injury at "drought" vs "optimum moisture" conditions was correlated with decreased stomatal conductance and gas uptake. However, Schramel did not quantify this relationship, nor were soil moisture levels between the extremes included. The high correlation coefficients obtained with our data indicate that all variables were correlated with decreased SO_2 injury. The available soil water would have a profound effect on all variables measured in our study. Limited soil moisture induces stomatal closure, which is known to inhibit SO_2 uptake and subsequent foliar injury in bean plants (14,17).

Transpiration, leaf water potential, and net photosynthesis are directly related to stomatal conductance (1,6,8). Increased stomatal opening and conductance rates have been reported at relatively low concentrations of SO_2 (2,3,12,13,24). However, stomatal conductance rate of fully watered plants may decrease in response to high concentrations of SO_2 (2,9,17). Sij and Swanson (20) reported that stomatal closure in response to SO_2 was caused by an increase in the partial pressure of CO_2 within the leaf caused by the inhibition of photosynthesis by SO_2. In our study, the conductance rate dropped rapidly with the onset of visible water-soaking, which was the first indication of macroscopic SO_2 injury. This decrease could have been the result of a reversible closure of the stomata or of irreversible closure caused by death of the cells near or comprising the stomata. The conductance rate of plants grown under -1 atm soil water potential increased after exposure when the plants were returned to the preexposure chamber, which suggests that in this case, the decrease in stomatal conductance was caused by a reversible closure of the stomata in response to the high concentration of SO_2.

Increased light intensity has been correlated with increased stomatal opening and increased conductance rates (4,8,21). The main environmental difference between our preexposure and exposure chambers was light intensity (33 and 45 klux, respectively). Moving the plants from a lower to a higher light intensity probably accounts for the large rise in conductance rate of the unexposed control plants at $-1/3$ and -1 atm (Fig. 4). This rise contrasts sharply with the large decrease in conductance rate in plants grown under the same soil moisture regimes but then exposed to SO_2 (Figs. 3 and 4) and emphasizes the role of SO_2 in inducing stomatal closure. Stomata of plants in -3 and -5 atm soil water potential did not show an increase in stomatal conductance rate when placed in the higher light intensity of the exposure chamber without SO_2, nor did their conductance rate decrease in response to SO_2. A lack of turgor in the guard cells of plants under water stress and the inability of the plant to build up turgor could explain the relatively consistent conductance rate of plants grown at -3 and -5 atm soil water potentials and exposed to light and SO_2.

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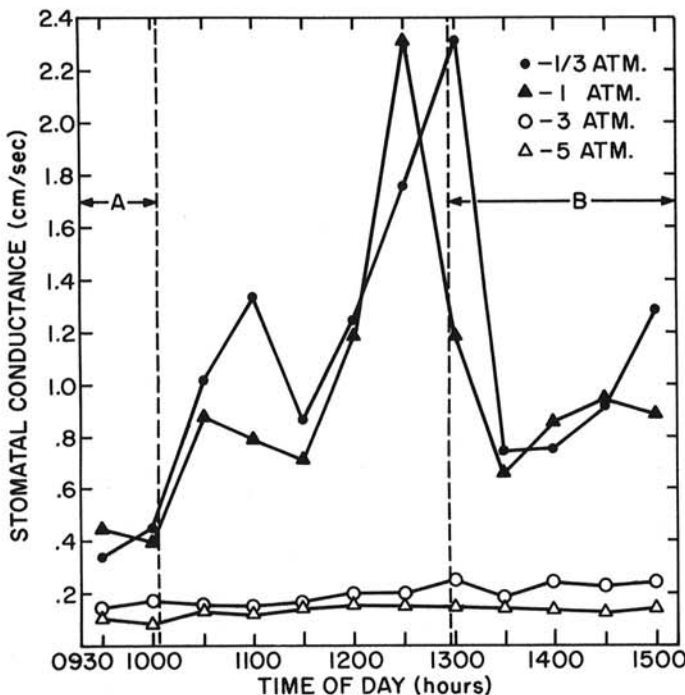25. Waring, R. H., and Cleary, B. D. 1967. Plant moisture stress:

Fig. 4. Stomatal conductance rates of the unifoliolate leaves of control pinto bean plants grown under varying soil water potential at 27 C, 75% relative humidity (RH), and 33 klux light intensity (A and B). Between the dashed lines, the plants were placed in the exposure chamber without SO_2 at 27 C, 75% RH, and 45 klux light intensity. Each point is the average of two replicates.

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