Sugar Content and Ozone Resistance in Water-Stressed Bean Plants

by

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ABSTRACT

The relationship of sugar levels to ozone resistance in 13-day-old primary leaves of water-stressed bean plants (Phaseolus vulgaris cv 'Pinto') was investigated. Solutions of 40%, 30%, 20%, 10%, 5%, 1%, and 0.1% (w/v) polyethylene glycol were used to induce water stress. Water stress was determined by measuring leaf diffusive resistance, osmotic potential, and water potential of 13-day-old primary leaves.

Two mechanisms for ozone resistance in bean plants were observed during the experiment, avoidance and repair of ozone injury. Plants treated with 10% and higher PEG concentrations had high leaf resistance values as compared to non-stressed plants indicating that the stressed plants closed their stomates. These plants were ozone resistant by avoidance, since ozone could not penetrate the leaf to cause damage.

Plants treated with 5% and lower PEG concentrations had open stomates as indicated by leaf resistance values. A decrease in osmotic potential and water potential was observed for all stress treatments. This indicated a rise in solute concentration of leaf cells. Subsequent ozone fumigation and sugar analysis showed that the 5% and lower PEG-treated plants were ozone resistant also and that at least a 25% rise in sugar levels over control plants occurred. Ozone resistance in these plants was considered an effect of increased sugar levels which probably allowed a repair mechanism for ozone injury to proceed.

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INTRODUCTION

Ozone is the most common phytotoxic air pollutant in the United States and causes considerable economic damage in both agronomic and horticultural crops (4,10,12,14). Damage is evident by bronzing, stippling or bleaching of the leaf (8,11). The extent of damage is dependent upon the ozone concentration, length of exposure time, and sensitivity of the plant.

Since ozone is a gas and must enter the plant through the stomates of leaves, stomatal activity is the rate-determining step for damage. Stomatal activity is controlled by several environmental factors, primarily light and moisture. Leone and Brennan showed that in begonias, high soil moisture levels favored injury when the plants were exposed to ozone (17). At the same time, they showed that stomatal pores were more open under wet conditions thus allowing more ozone to enter the leaf (17). Heck and Dunning found that Pinto bean plants were more sensitive to ozone at midday, probably because of light-induced stomatal opening (9).

Various environmental factors also cause stomatal closure. Many plants respond to severe water stress by stomatal closure. Thus, plants experiencing water stress will tend to be ozone resistant since the closed stomates prevent ozone penetration. However, plants under mild water stress or plants

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The format and style of this thesis will follow that of Plant Physiology .

adapted to water stress do not always respond by stomatal closure.

Ackerson and Hebert found that water-stress-adapted cotton plants did not have increased leaf resistance, the parameter used to measure stomatal activity, indicating that stomatal activity was unchanged from non-stressed plants (2). They found that in the adapted plants, carbohydrate levels of the leaves increased (1). Amthor, et. al., found that plants treated with Treflan, a commonly used herbicide, showed a similar increase in carbohydrate levels with no increase in leaf diffusive resistance (3). They also showed that Treflan-treated plants had a higher shoot to root ratio than non-treated plants (3). This higher shoot to root ratio could cause the plant to undergo mild water stress.

It has been suggested that ozone resistance might be a function of carbohydrate levels in the leaves (5,6,7,13). Hull and Went found that plants depleted of carbohydrates were more susceptible to ozone (13). Dugger, et. al., while working with Pinto bean plants, obtained data suggesting that sugar levels were related to ozone sensitivity (5,6,7). Amthor, et. al., also found that the Treflan-treated plants were ozone resistant, and he suggested that this was due to the increase in sugar levels prior to ozone fumigation (3). In this paper, our work with water-stressed bean plants supports previous suggestions that sugar levels in leaves are related to ozone resistance.

MATERIALS AND METHODS

Plant Material. Pinto beans (Phaseolus vulgaris cv 'Pinto') were grown from seed in vermiculite in 400 ml styrofoam cups. Plants were kept in a growth chamber under the following environmental conditions: irradiance, 225 $uE \cdot m^{-2} \cdot s^{-1}$ supplied by a mixture of cool-white fluorescent and incandescent lamps; photoperiod, 12 h light/12 h dark; temperature, 26 to 32C light/18 to 21C dark; humidity, 35 to 50% day/90 to 100% night. Plants were watered daily with quarter-strength Hoagland's solution until stress treatments began. On the 7th day after planting, plants were thinned to one per cup. All procedures were conducted on plants 8 to 13 days old. Age was determined from the day of planting.

Stress Treatments. Polyethylene glycol (PEG, mw 8000) was used to induce water stress. On the 8th, 10th, and 12th days after planting, 50 ml of 40%, 30%, 20%, 10%, 5%, 1%, or 0.1% (w/100 ml distilled H_2O) PEG solutions were applied to the vermiculite of bean plants. The treated plants were not watered on the 9th and 11th days after planting. Controls were watered daily through the 12th day after planting with quarter-strength Hoagland's solution.

Leaf Diffusive Resistance. Leaf diffusive resistance was measured using a calibrated Lambda diffusive resistance meter (Licor model LI-60) and sensor (LI-20s). Calibration was done in the growth chamber as described by Kanemasu, et. al.(16).

Readings were taken using the abaxial surface of primary leaves after plants were exposed to 3 to 4 h of light. Plants were not watered before readings were taken.

To determine if clamping the sensor onto the leaf affected the leaf diffusive resistance, the leaf resistances of primary leaves were repeatedly measured on a daily basis on days 8 through 12. Separate sets of plants were measured on days 9 through 12, or on days 10 through 12, or on days 11 through 12, or on the 12th day.

Osmotic Potential. Osmotic potentials were determined psychometrically on leaf discs using isopiestic thermocouple psychometry as described by Ackerson and Hebert (2). A Wescor model HR33T microvoltmeter with C-52 chamber was used. Leaf tissue was frozen in liquid N_2 for 10 min and thawed for 30 min. After thawing, 8 mm discs were cut directly next to but not including the midvein and between the first and second major lateral veins of leaf tissue. Previously, it was determined in the lab that this area of the leaf was the least variable for osmotic potentials between different leaves on different plants as well as on the same leaf. Equilibration time in the thermocouple was 10 min. Eight readings, 2 leaf discs per primary leaf, 2 leaves per plant, 2 plants per stress treatment and control, were averaged to obtain the final osmotic potential value for each treatment. All readings were taken on leaf tissue 13 days after planting.

Water Potential. Water potentials of primary leaves were determined using the pressure bomb technique described

by Scholander, et. al. (18). All measurements were made after plants were exposed to light for 3 to 4 h. Plants were not watered on the day measurements were taken. Four readings per treatment were taken and averaged to obtain final water potential values in negative bars.

Ozone Fumigation. Plants treated with 40%, 5%, 1%, and 0.1% (w/v) PEG and control plants were fumigated with ozone in the growth chamber. Ozone was generated by passing charcoal-filtered air over a 150-watt Conrad ultraviolet light lamp. Ozone concentration was controlled by adjusting the voltage to the lamp. Actual ozone concentration in the growth chamber was not monitored. Plants were fumigated with ozone for 2 to 2½ h. Plants were fumigated when most sensitive to ozone, after 3 to 4 h of light exposure on the 13th day after planting (8,9). Three plants were fumigated for each stress treatment and control treatment. Three separate fumigations were done. The first time, controls and 40% PEG-treated plants were fumigated. Controls and 5% PEG-treated plants were fumigated the second time. The third time, controls, 0.1% PEG-treated plants, and 1% PEG-treated plants were fumigated.

Injury was scored on the 14th day by comparing the amount of damage to the leaf surface of primary leaves of stressed plants to the amount of damage to the leaf surface of primary leaves of control plants. Damage was determined by a bronzed appearance on the adaxial leaf surface (8,11). Primary leaves of PEG-treated plants with less than 10%

injury to the leaf surface as compared to control plants were classified as resistant.

Sugar Analysis. The primary leaves of plants treated with 5%, 1%, and 0.1% (w/v) PEG and control plants were used for carbohydrate analysis. Both primary leaves of each plant were removed after 3 to 4 h of light on the 13th day after planting. Three pairs of primary leaves were analyzed per treatment. The leaves were weighed and then ground to a powder in liquid nitrogen. The powder was boiled in 100 ml of distilled water for 1½ h to extract sugars. After boiling, the mixture was filtered while hot using a Buchner funnel. The filtrate was resuspended to 140 ml. A 40 ml aliquot was frozen for future analysis.

The frozen samples were thawed to room temperature. The carbohydrate levels were determined by the anthrone method described by Yemm and Willis using glucose as a standard (20). The results were expressed as mg of glucose per g fresh weight.

RESULTS

Plants treated with 30% and 40% (w/v) PEG were stunted in height compared to control plants. The primary leaves of 40% PEG-treated plants were smaller than those of control plants. The size of primary leaves on 30% PEG-treated plants was not affected. No stunting was apparent for 20% and lower PEG treatments. The fresh weights of primary leaves from 0.1%, 1%, and 5% PEG-treated plants were recorded and compared to the fresh weight of primary leaves from control plants (Table I). No significant difference existed thus supporting the visual observation that no stunting was occurring in plants treated with lower PEG concentrations.

Resistance of leaves measured repeatedly did not significantly increase, but the variability of readings increased from the 8th to the 12th day (Figure 1). In view of the increased variability, later readings were done by measuring the leaves only one time on a different plant each day rather than measuring the same leaf each day over a period of days.

The effect of leaf age on leaf diffusive resistance for various PEG treatments is shown in Figure 2. Only six PEG treatments were used for this procedure; the 40% PEG treatment was omitted. For the 30%, 20%, and 10% (w/v) PEG treatments, leaf resistance increased with leaf age. The values for day 13 in all three cases are significantly higher than the control value which was 0.7 ± 0.5 sec·cm^{-1} on day 13. The

Table I. Effect of Polyethylene Glycol on the Fresh Weight of Primary Leaves 13 Days After Planting

PEG Treatment (% w/v)	Fresh Weight* (FW ± S.D. in gms)
Exp. 1[1]	
Control 0%	2.1 ± 0.4
PEG 5%	1.7 ± 0.3
Exp. 2[1]	
Control 0%	2.0 ± 0.6
PEG 1%	1.7 ± 0.4
PEG 0.1%	1.6 ± 0.3

*Value is the average of 3 leaf pairs.

[1]Fumigations for these PEG treatments were done on two different dates. A new set of controls was used each time.

Fig. 1. Relationship of frequency of porometer measurements and leaf resistance values on 12-day-old primary leaves of bean plants. Each data point is the mean of four readings, 2 leaves per plant, 2 plants per number of porometer measurements. Bars represent the standard deviation.

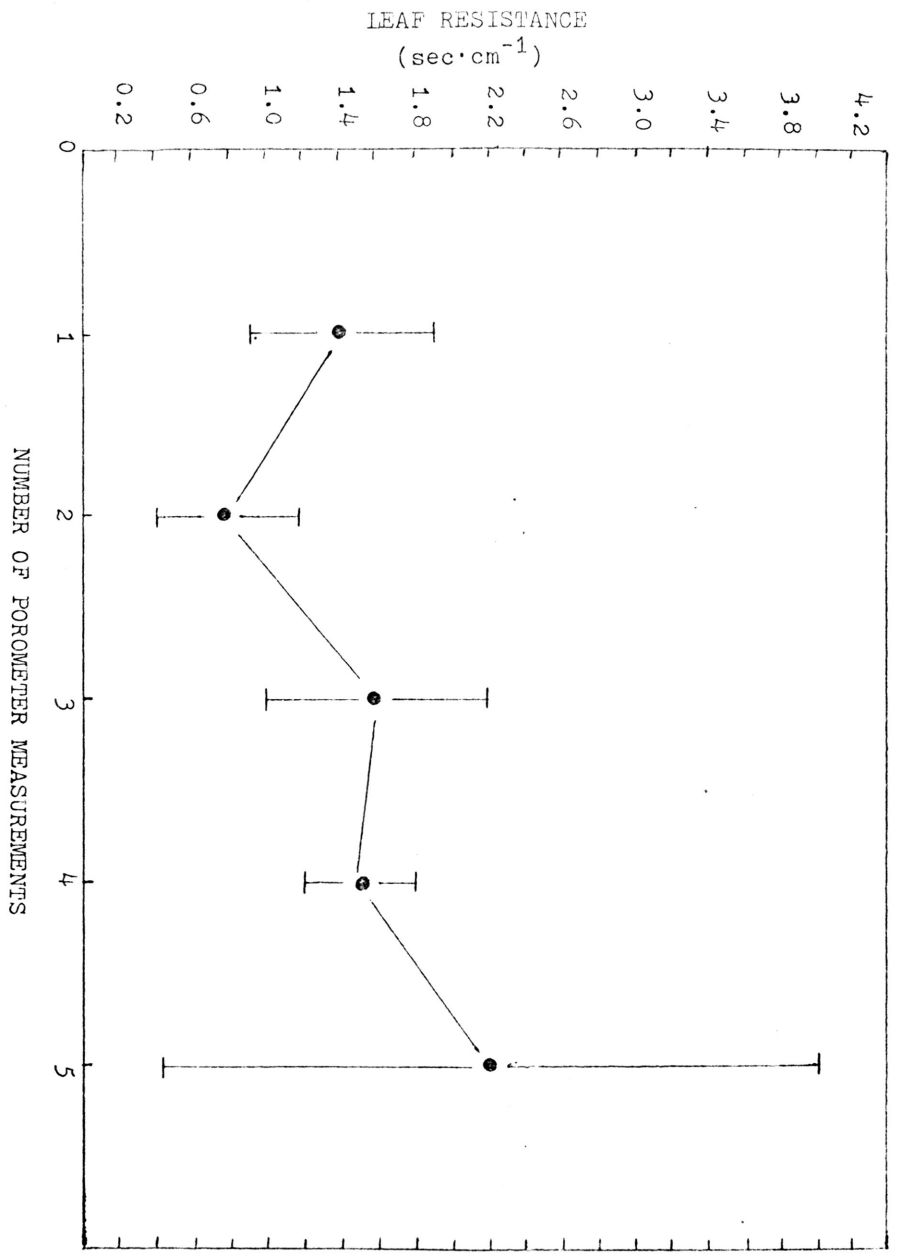
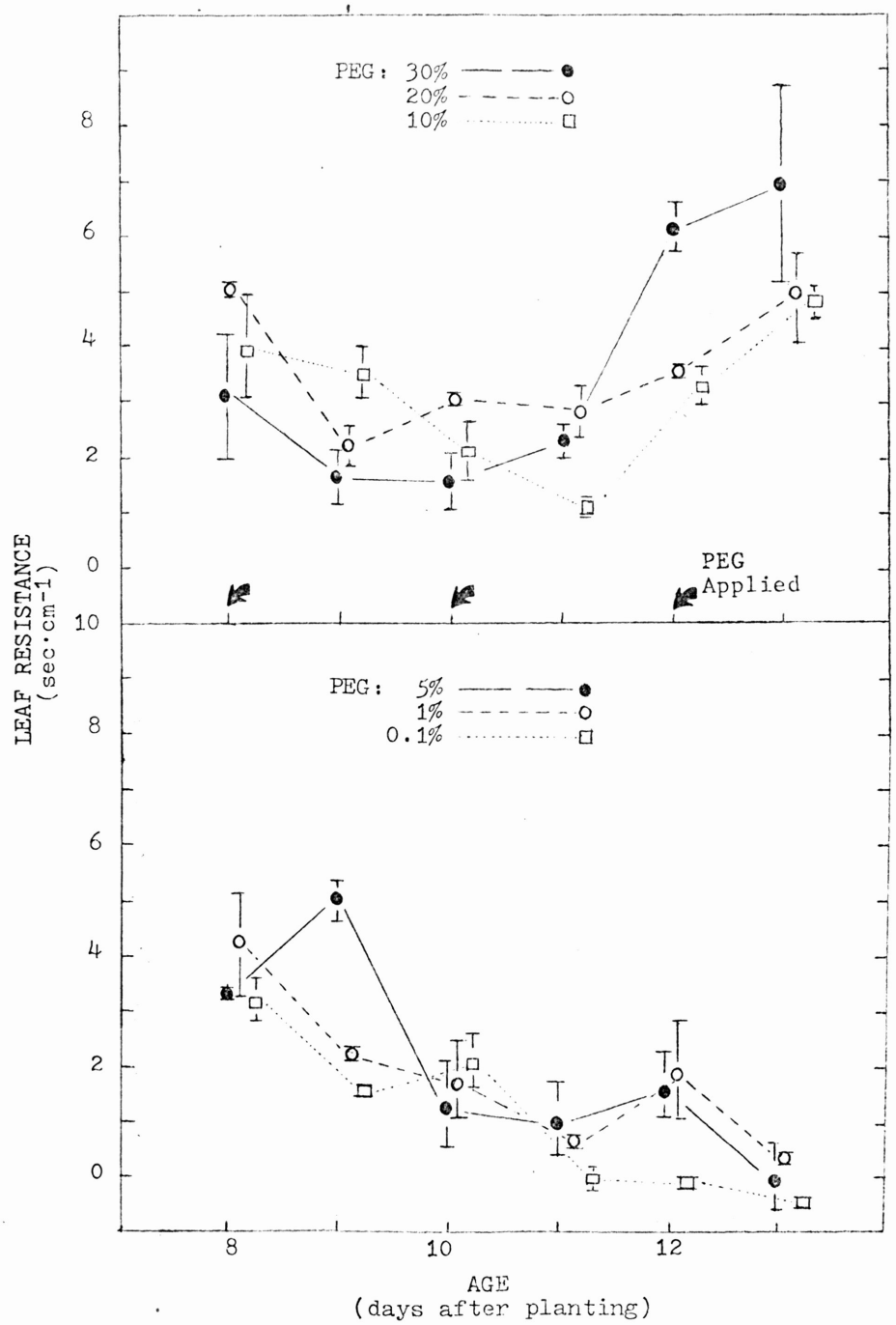

LEAF RESISTANCE
(sec·cm^{-1})
0.2
0.6
1.0
1.4
1.8
2.2
2.6
3.0
3.4
3.8
4.2
0
1
2
3
4
5
NUMBER OF POROMETER MEASUREMENTS

Fig. 2. Relationship of primary leaf age to leaf resistance values at six different polyethylene glycol concentrations. Leaf resistance values for plants treated with 30%, 20%, and 10% (w/v) PEG are represented in the upper graph. Leaf resistance values for plants treated with 5%, 1%, and 0.1% PEG are shown in the lower graph. PEG was applied on the 8th, 10th, and 12th days after planting. Control values for days 8 and 13 were 4.4 ± 0.1 $sec \cdot cm^{-1}$ and 0.7 ± 0.5 $sec \cdot cm^{-1}$, respectively. All data points are the mean of two readings, ie., 2 leaves per plant, 1 plant per treatment per day. Bars represent standard deviation.


PEG: 30%
20%
10%
PEG: 5%
1%
0.1%
PEG Applied
LEAF RESISTANCE (sec·cm-1)
AGE (days after planting)
0
2
4
6
8
10
12

control value for day 8 was 4.4 ± 0.1 $sec \cdot cm^{-1}$.

For the 5%, 1%, and 0.1% (w/v) PEG treatments, leaf diffusive resistance decreased with leaf age in a fashion similar to the control plants. No significant difference was noted between the resistance values for the control plants and those for the 5%, 1%, and 0.1% PEG-treated plants.

Since plants were fumigated on the 13th day after planting, the values for day 13 for each PEG treatment were replotted in Figure 3 so that leaf resistance was compared to PEG concentration. The 10%, 20%, and 30% (w/v) PEG-treated plants had leaf resistances significantly higher than the control plants. The leaf resistances for 0.1%, 1%, and 5% PEG-treated plants were not significantly different from the control plants. The results, as seen in Figures 2 and 3, indicate that at the 10%, 20%, and 30% PEG levels, treated plants responded to stress by stomatal closure thus causing the leaf resistance to increase. For the 0.1%, 1%, and 5% PEG treatments, leaf resistance did not increase indicating that the plants did not respond to stress by stomatal closure.

Osmotic potentials of 13-day-old primary leaves became more negative with increased PEG concentration (Figure 4). Very little difference was noted among the 0.1%, 1%, 5%, and 10% (w/v) PEG treatments, but the 20%, 30%, and 40% PEG treatments were progressively more negative. The osmotic potentials for all treatments, even the lowest at 0.1% PEG, were significantly more negative than the control. This indicates that for all stress treatments, the plants responded

Fig. 3. Relationship of polyethylene glycol concentration to leaf resistance values in 13-day-old primary leaves. Data points are the mean of two readings, 2 leaves per plant, 1 plant per treatment per day. Bars represent standard deviation.

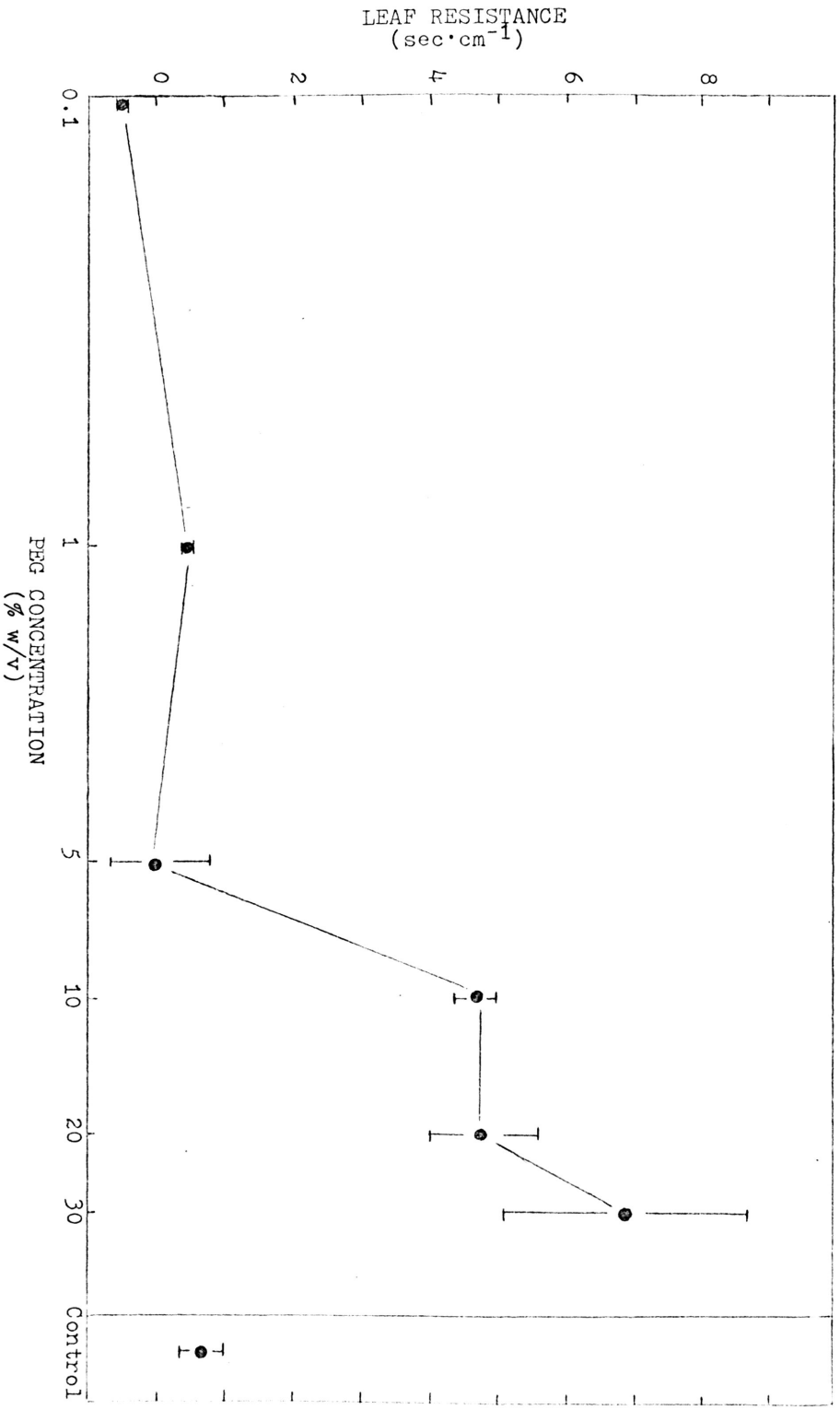
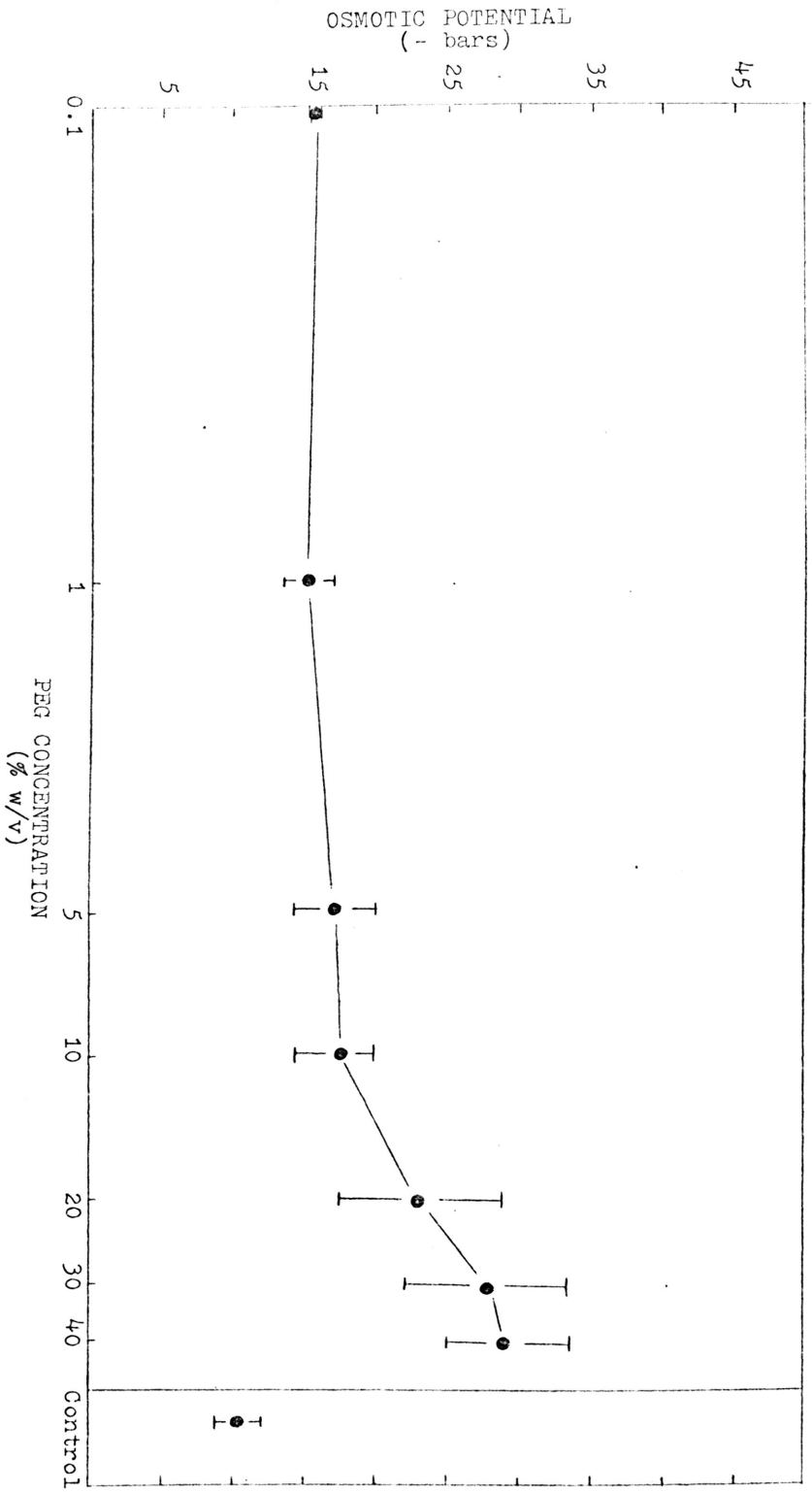

LEAF RESISTANCE
(sec·cm⁻¹)
0
2
4
6
8
0.1
1
5
10
20
30
Control
PEG CONCENTRATION
(% w/v)

Fig. 4. Relationship of polyethylene glycol concentration to osmotic potential of 13-day-old primary leaves. Data points are the mean of eight readings, 2 leaf discs per leaf, 2 leaves per plant, 2 plants per PEG treatment. Bars represent the standard deviation.


OSMOTIC POTENTIAL
(- bars)
5
15
25
35
45
PEG CONCENTRATION
(% w/v)
0.1
1
5
10
20
30
40
Control

with a rise in solute concentration.

Water potential of primary leaves did not change significantly from 8 to 12 days after planting for control plants; however, on the 13th day, the water potential was significantly more positive (Figure 5). The water potential of 13-day-old primary leaves of plants treated with various PEG concentrations were significantly more negative than the water potentials of primary leaves of control plants (Figure 6). Water potential data was expected to follow the trend obtained with osmotic potentials since the two parameters are related. However, this was not the case. The data showed that the plants were undergoing water stress. Possibly, the data also shows that the plants are adapting in some way to the water stress (15).

Ozone injury was evident by the bronzed appearance of interveinal tissue on the upper surface of primary leaves of control plants (8,11). No apparent injury was seen on the leaves of 40% (w/v) PEG-treated plants. Plants treated with 0.1%, 1%, and 5% PEG had less than 10% injury as compared to controls and were regarded as resistant (Table II). Plants treated with 10%, 20%, and 30% PEG were not fumigated.

Sugar levels of 0.1%, 1%, and 5% (w/v) PEG-treated plants and control plants were measured. In all cases, the sugar levels of PEG-treated plants were higher than control plants (Table III). Since 5% PEG-treated plants were fumigated on a separate date from 0.1% and 1% PEG-treated plants, two different sets of controls are represented in Table III. The PEG-treated plants are compared to their respective control.

Fig. 5. Relationship of primary leaf age to water potential. Plants were watered daily with quarter-strength Hoagland's solution. Data points are the mean of four readings, 2 leaves per plant, 2 plants per daily measurement. Bars represent standard deviation.

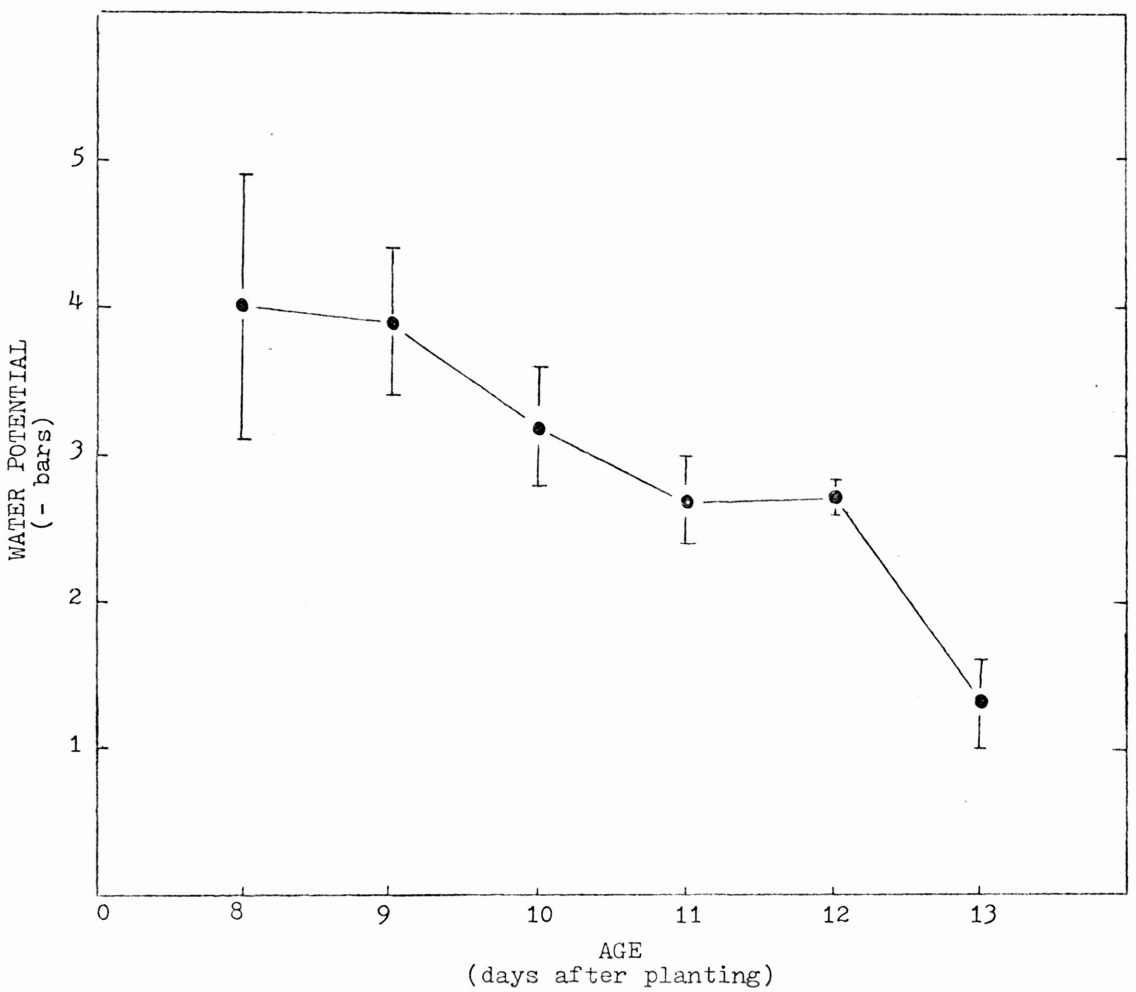


Fig. 6. Relationship of polyethylene glycol concentration to water potential of 13-day-old bean plants. Data points are the mean of four readings from four different leaves, 2 leaves per plant, 2 plants per treatment. Bars represent standard deviation.

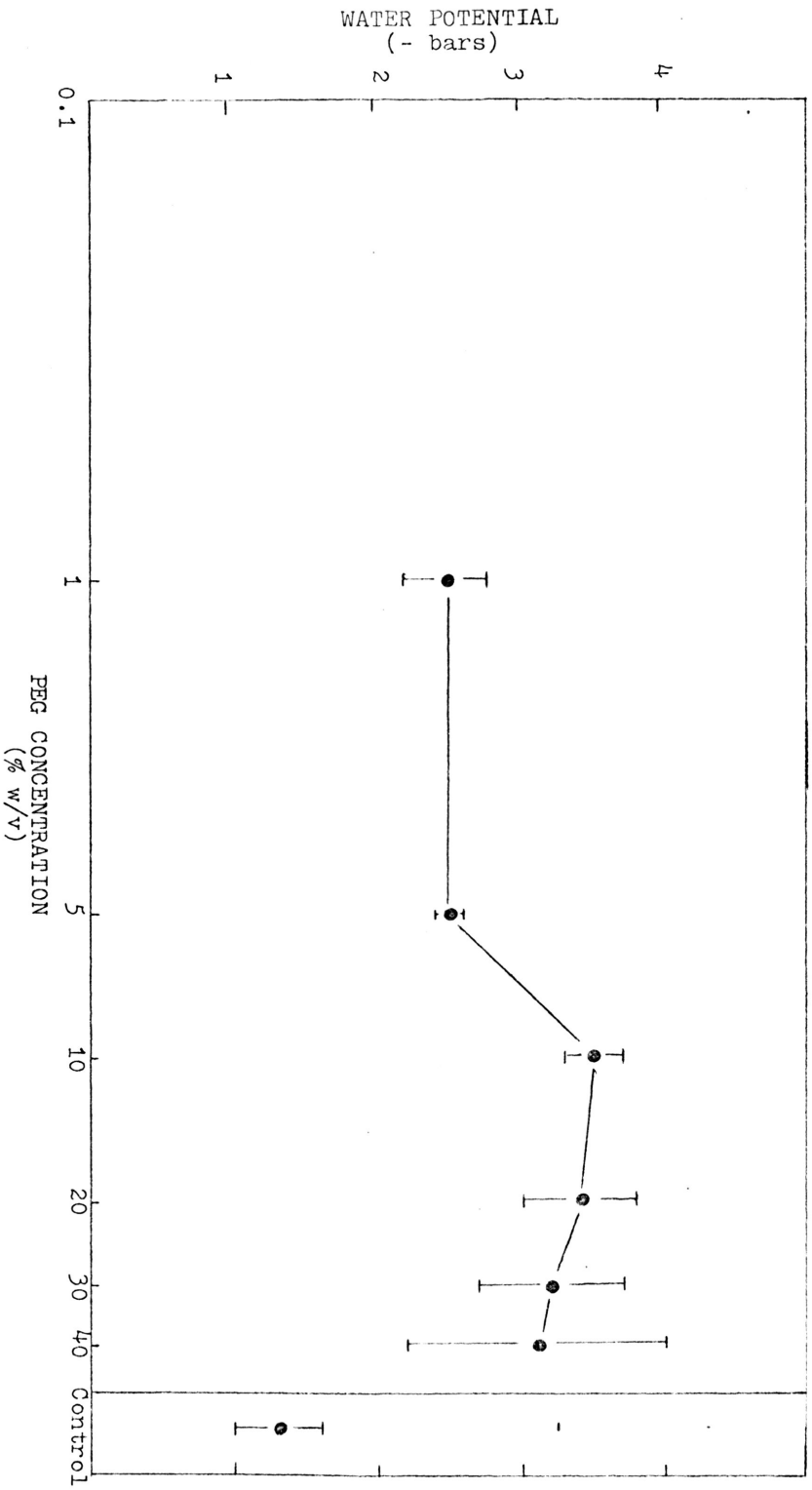

WATER POTENTIAL
(- bars)
1
2
3
4
0.1
1
5
10
20
30
40
Control
PEG CONCENTRATION
(% w/v)

Table II. Effect of Polyethylene Glycol on Ozone Injury in 13-Day-Old Bean Leaves

PEG Treatment (% w/v)	Leaf Injury*
Exp. 1*	
Control 0%	90% - 100%
PEG 40%	No apparent injury
Exp. 2*	
Control 0%	90% - 100%
PEG 5%	1% - 10%
Exp. 3*	
Control 0%	90% - 100%
PEG 1%	1% - 10%
PEG 0.1%	1% - 10%

*Fumigations were done on three different dates. Controls were fumigated each time as well as the designated PEG-treated plant. Leaf injury was recorded as % of leaf area damaged. Control plants were given the range of 90% to 100% injury. The PEG-treated plants were scored as to the amount of leaf area that was damaged in comparison with the control they were fumigated with. Plants were regarded as resistant if leaf injury was between 1% and 10%.

Table III. Sugar Content of 13-Day-Old Bean Leaves

PEG Treatment (% w/v)	Sugar Content (mg/g FW ± S.D.)	% Increased Sugar (PEG treated/Control)
Exp. 1*		
Control 0%	4.9 ± 0.4	
PEG 5%	8.9 ± 2.3	182
Exp. 2*		
Control 0%	0.8 ± 0.3	
PEG 1%	1.0 ± 0.3	125
PEG 0.1%	1.6 ± 0.1	200

*Analyses were done for two different sets of plants. Comparisons of PEG-treated plants and controls are kept within the respective groups.

When the ratio of sugar levels in PEG-treated plants to sugar levels in control plants is calculated, it appears that ozone resistance is apparent when a 25% to 100% increase in sugar levels occurs in the leaves (Table III).

DISCUSSION

Three mechanisms have been proposed to explain ozone tolerance in higher plants: avoidance, detoxification, and repair. Avoidance mechanisms involve the closure of stomates to prevent any toxic gases from entering the sensitive mesophyll tissue of the leaf. Detoxification mechanisms involve some process by which ozone is converted to a non-toxic specie. Finally, repair of ozone injured or damaged cellular constituents by the plant is possible (19).

In plants which are water-stressed over three days with polyethylene glycol (PEG), we have found at least two different mechanisms of ozone tolerance induced in bean plants. With 10% (w/v) or higher PEG concentrations, the leaf resistances, an indicator of gas movement through a leaf, are higher in comparison to leaves of non-stressed controls (Figure 3). The high leaf resistances indicate the stomates are closed, and gases such as ozone will not penetrate into the leaf interior.

In contrast, plants treated with 5% (w/v) or lower PEG concentrations have no significant change in their leaf resistances from the values of plants not water-stressed with PEG (Figure 3). These leaves have open stomates like the controls.

Even though stomatal closure isn't apparent until PEG concentrations as high as 10% (w/v) are used, ozone tolerance is evident for all bean plants water-stressed with any concentration of PEG (0.1% to 40%) (Table II). This strongly

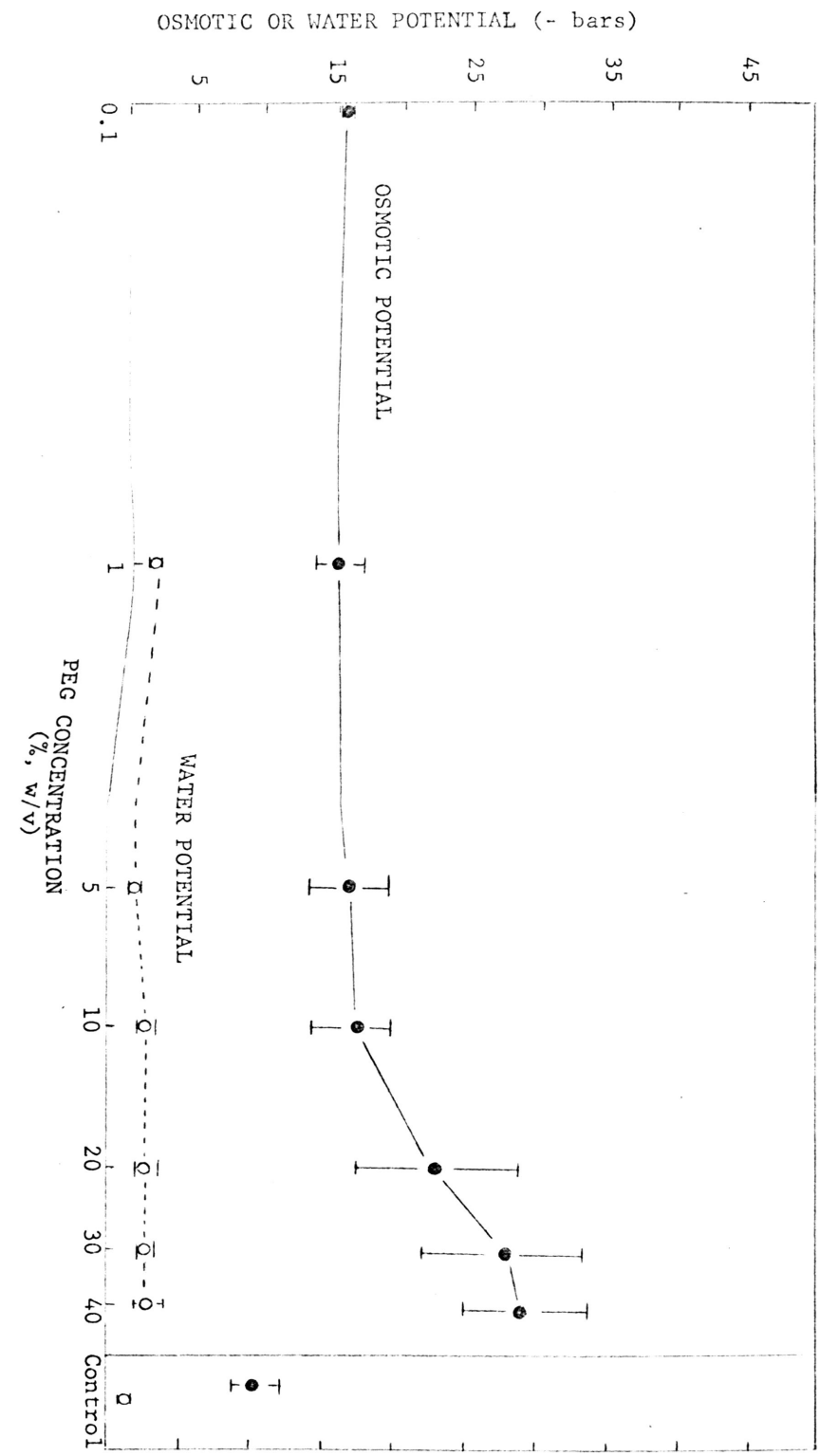
suggests that some other mechanism for ozone tolerance is being used in plants stressed with a 5% or lower PEG concentration.

Other indicators of water stress are osmotic potential and water potential. In all PEG treatments, it was shown that the osmotic potentials were significantly more negative than controls indicating a rise in solute concentration (Figure 4). Water potentials of PEG-treated plants also became more negative (Figure 6), but the change in water potential was not as pronounced as the change in osmotic potentials (Figure 7). This data suggest that the treated plants are adapting to the induced water-stress by some degree of osmotic adjustment to maintain a relatively constant water potential (15).

The water-soluble carbohydrate content of water-stressed leaves was found to be significantly higher in all PEG-treated plants where stomatal closure wasn't a factor in ozone resistance (Table III). Increases in carbohydrate content has been reported in cotton leaves which are under water stress (1). This increase in carbohydrate content may be caused by increased amylase activity which degrades starches, or by changes in the loading and unloading of sugars in the phloem tissue (1). The increase in soluble carbohydrates in water-stressed bean leaves probably accounts for the more negative osmotic potential of the leaves.

Sutton and Ting surmised from experimental evidence that glucose probably acts by providing the energy necessary to repair ozone injured cell components (19). Amthor, et. al., found that application of the herbicide Treflan caused a rise

Fig. 7. Comparison between the relationships of osmotic potential (●——) and water potential (○ - - -) of 13-day-old bean leaves to polyethylene glycol concentration. The values are the same as those plotted in Fig. 4 (osmotic potential) and in Fig. 6 (water potential).


OSMOTIC OR WATER POTENTIAL (- bars)
5
15
25
35
45
OSMOTIC POTENTIAL
WATER POTENTIAL
0.1
1
5
10
20
30
40
Control
PEG CONCENTRATION
(%, w/v)

in carbohydrate levels and at the same time conferred ozone resistance in bean plants (3). The results reported here show that PEG-induced water stress will cause an increase in soluble carbohydrates resulting in increased ozone tolerance. Thus, our results are consistent with the hypothesis that the availability of sugars is one important factor in ozone tolerance.

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