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PHYSIOLOGICAL RESPONSE OF PLANTS TO SALINITY

by

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I hereby recommend that this dissertation prepared under my direction by **James J. Riley** entitled **Physiological Response of Plants to Salinity** be accepted as fulfilling the dissertation requirement of the degree of **Doctor of Philosophy**

James W. O'Leary — 11/17/67
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ACKNOWLEDGMENTS

On November 3, 1949, Sir Winston Churchill was quoted by The Times of London as saying: "Broadly speaking, short words are best, and the old words, when short, are the best of all." I would like to record here a few short, and some old, words for those who have made this project possible. I am indebted to Dr. A. Richard Kassander, Jr., for his interest in utilization of sea water which stimulated this project, and for his continued support throughout its duration. I want to express my most sincere appreciation to Carl N. Hodges and the members of the Environmental Research Laboratory for their invaluable assistance. For his help as advisor and co-researcher, I owe special thanks to Dr. James W. O'Leary. It is seldom one has the privilege of working with a colleague as interesting or as stimulating as Jose Gerrado Besera Oliveira. I want to thank him for the many fruitful discussions and his hard labor in the greenhouse.

On the same occasion as mentioned above Sir Winston Churchill was asked about writing a book. He replied: "Writing a book was an adventure. To begin with it was a toy, an amusement; then it became a mistress, and then a master, and then a tyrant, and the last phase was that, just as one was about to be reconciled to one's servitude, one killed the monster."

I want to thank the following workers for the many hours they labored on this project to kill the monster: Newton Don, Richard Pearce, David Evans, Fred Zinowich, Linda Mariani, and Sandy Kramer. I especially want to thank Linda and Sandy who knew the full wrath of the monster of data analysis.

It is customary at this point to thank your wife and your family and I do not intend to depart from this custom. Thank you Josie, Christine, and Gabrielle. However, I think one must say more since the devotion of time for the attainment of an advanced degree necessarily means a detachment from normal family duties. This is a particularly high price for a family to pay--but it has been paid. There is no way this time can be recaptured, but I would like to have them recall John F. Kennedy's words in his inaugural address: "Here on earth God's work must truly be our own."

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ABSTRACT

Plants growing under saline conditions exhibit symptoms resembling the symptoms of drought. Results showing osmotic adjustment of the sap of plants treated with NaCl have led to the assumption that the water potential of the plants also adjusts. Observations of leaf water potentials of red kidney beans (*Phaseolus vulgaris* L.) in this study show that this assumption is not valid. The gradient of water potential from the growth solution to the leaves decreased for plants growing in nutrient culture with NaCl or $CaCl_2$ added while plants treated with Carbowax 1540 made a nearly complete adjustment and plants treated with Na_2SO_4 a partial adjustment. In addition, plants in all the treatments showed an increase in the resistance to water movement through the roots which was accompanied by morphological changes in the root structure. These factors combined to produce a water stress in the leaves which resulted in partial stomatal closure and an increase in the resistance to vapor diffusion from the leaves. The transpiration and photosynthetic rates were reduced. The decrease in water uptake almost entirely explains the decrease in the uptake of Na, K, Ca and Mg ions from the growth solution. However, the ion concentration in the water uptake stream was not equal to the ion concentration in the nutrient solution.

CHAPTER 1

INTRODUCTION

1.1 Water as a Resource

Low quality grades of most resources are usually the most abundant simply because of the unique conditions required to produce high concentrations of pure substance (1). Water in the context of a resource is no exception. Ninety-seven percent of the total water on earth is in the form of sea water, which has 34, 000 parts per million (ppm) of dissolved solids (78), (32). In addition, it is estimated that two-thirds of the continental United States is underlain at shallow depths with water containing 1000 ppm or more of dissolved solids (18). Thus, our endowment of pure water is limited. Projections for the future indicate that scarcity of pure water will be one of the most serious problems facing us in the next few years. Recognition of the water shortage problem is difficult to perceive in some areas since depletion of the pure water supply is not abrupt, but rather takes the form of general resource scarcity as described by Scott (61): "... resources do not just disappear, but, as the highest grade stocks are consumed, lower grades take their place, requiring higher costs of exploitation or refining." In addition, pollution causes a degradation of high quality water, thus reducing still further the pure water supply.

Lower grade water supplies are already being used where high quality water is not essential, such as for cooling of condensers (67). However, water to be used to satisfy domestic drinking requirements must be of high quality. Only the highest grade natural reservoirs of water can fulfill the specific quality requirements of the U.S. Public Health Service as shown in Table I (72). Thus, the natural supply of high quality water will be used in preference to lower quality water to meet the needs of large urban population centers, Only the water of lower quality will remain to fulfill other needs such as irrigation of agricultural crops.

In 1960, eighty percent of all the water removed from ground water reservoirs in the United States was used for irrigation of crops (37). In 1955, it was estimated that more than one-fourth of all the irrigated lands in the U. S. experienced some decrease in productivity due to saline or alkaline conditions (8). As the quality of water available for irrigation decreases, the number of acres experiencing decreased yields will increase. In addition, with current irrigation practices, more of the low quality water will have to be applied in order to prevent salt accumulation. Thus, at a time when more tons of food per acre are required, the utilization of lower quality water will not only result in less production per acre, but will require more water. In areas, such as the West, where population increases have put a strain on the water budget, many propose irrigation should be decreased;

TABLE I

U. S. PUBLIC HEALTH SERVICE
DRINKING WATER REQUIREMENTS (72)

Ion	Supply Rejection Limit (ppm)	Primary Reason
Arsenic	0.05	Causes skin and lung cancer
Barium	1.00	Causes high blood pressure
Cadmium	0.01	Retards growth
Chromium	0.05	Carcinogenic agent
Cyanide	0.20	Toxic to fish
Fluoride	0.80	Causes dental fluorosis
Lead	0.05	Cumulative poison
Selenium	0.01	Carcinogenic agent
Silver	0.05	Causes skin discoloration

Ion	Recommended Limit (ppm)	Primary Reason
Chloride	250	Laxative properties
Copper	1.0	Taste
Iron	0.30	Brownish color
Manganese	0.05	Potential stimulant of hepatic cirrhosis
Nitrate	45	Associated with sewage
Sulfate	250	Laxative properties
Zinc	5	Milky color
Total dissolved solids	500	---

but this poses other problems as outlined by Landsberg (37) in 1964: "If it seems best for an area as a whole to shift some water from agriculture to other uses, what about the families who have to leave the farms; and what about the small towns whose main function has been to service irrigated farm country?"

To help mitigate the phasing out of agriculture, ways must be found to maintain high yields with either less water or with water of low quality. In order to increase the yield of crops irrigated with brackish water, one must first understand the reasons why plant growth is inhibited by brackish water; then one can make the necessary modifications in the plants, the water, the irrigation procedures, or in the environment which will allow full utilization of all the available water resources.

1.2 Plant Water Relations

Most plants are composed of 85 to 95 percent water. Water enters into vital metabolic reactions such as photosynthesis and serves as a solvent for many other reactions. In its movement through the plant, water transports solutes from the roots to the leaves and other growing regions of the plant (58). It is the latter role that will be considered in greatest detail in this study.

Water moves in the soil and in plants in response to energy gradients. It moves from regions of high free energy to regions of low free energy. The free energy of pure water is decreased by the addition of solutes and increased by the application of pressure. The pressure required to maintain water containing solutes at the same free energy level as pure water at the same temperature is called the osmotic pressure, or preferably the osmotic potential (12). Plant cells have hydrostatic pressures above atmospheric levels. It is not far from the truth to think of a healthy plant cell as being like a water-filled balloon; that is, it is held up by an internal hydrostatic pressure often referred to as the turgor pressure. A plant wilts because it loses water from the leaves faster than it can be supplied by the roots. The wilting of a plant cell is like the collapse of a balloon with a leak. The total water potential in a plant is defined as the difference between the pressure potential and the osmotic potential as shown in Equation 1.1 (35).

$$\Psi = P - \Pi \qquad 1.1$$

Where Ψ = total water potential (atm)

P = pressure potential (atm)

Π = osmotic potential (atm)

The total water potential is equivalent to the free energy difference between the water in the cell and pure water at the same temperature, expressed as a ratio to molar volume (35). It has the units of $\frac{ergs}{cm^3}$. Since $10^6 \frac{ergs}{cm^3} = 10^6 \frac{dynes}{cm^2} = 0.987$ atmospheres, the units are usually expressed in atmospheres (66). For example, a cell with a hydrostatic pressure of two atmospheres and an osmotic potential of four atmospheres would have a total water potential of minus two atmospheres. If this cell were in contact with pure water, at the same temperature, water would flow into the cell since the free energy is below that of pure water.

The driving gradient, or the water potential gradient, is not the only factor that governs water movement through a plant. The resistance, or the inverse, the permeability of the conducting tissue to water movement also must be considered. Water entering the root passes through the endodermis to reach the conducting tissue, the xylem, through which water is transported throughout the plant. The water reaching the stomatal cavities of the leaves is lost to the atmosphere by vapor diffusion. Each of the resistances to water movement throughout the system is important since the highest resistance will limit the rate of water movement through the whole system (33).

1.3 Salinity and Plant Growth

1.31 Osmotic Effects. The yield of crops has been found to decrease as the concentration of dissolved solids in the irrigation water increases. Figure 1 shows a typical response of plants irrigated with saline water (43). The accepted explanation for the decrease in growth and yield caused by saline conditions was, for many years, "physiological drought," which was described by Harris in 1924 (21) as: "The... incapacity of agricultural plants for developing osmotic concentrations sufficiently high to enable them to withdraw water from the soil."

As early as 1919 Lundegardh (42) presented data showing that plants irrigated with sodium chloride enriched nutrient solutions increased the salt content of their sap in proportion to the level of sodium chloride in the irrigation water. But not all researchers have found a sufficient adjustment in osmotic concentration to prevent physiological drought (3). Bernstein (4) concluded in 1961 that data showing an apparent lack of osmotic adjustment of root sap, under saline conditions, were probably due to the standard procedure of rinsing the roots in distilled water prior to obtaining the sap from them. He reasoned that rinsing with distilled water tended to dilute the concentration of the sap obtained. He presented data for cotton plants irrigated with sodium chloride enriched water which showed roots not rinsed have nearly complete osmotic adjustment. (See Table II) Notice that the leaves also made a nearly complete osmotic adjustment.

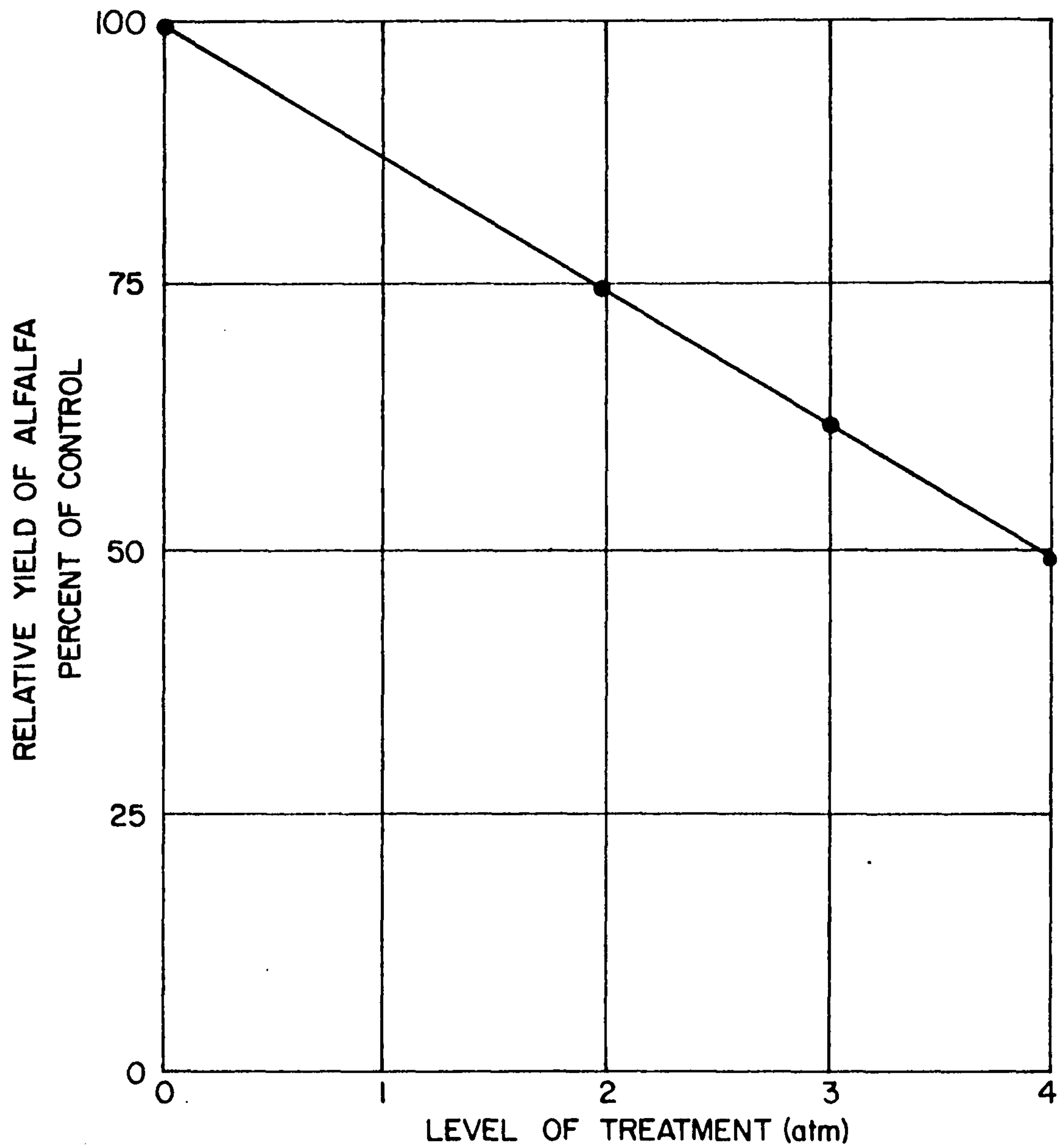

Fig. 1.--Relative Yield of Alfalfa in Sand Cultures of Varied Osmotic Pressure (Data from Magistad (43))

TABLE II

OSMOTIC ADJUSTMENT OF SAP EXPRESSED FROM COTTON PLANTS (4)

Treatment Level of NaCl (atm)	Osmotic Potential (atm)					
	Rinsed Roots		Unrinsed Roots		Leaves	
	π	$\Delta\pi$	π	$\Delta\pi$	π	$\Delta\pi$
0	5.6	5.2	4.9	4.5	10.2	9.8
3	8.6	5.2	8.8	5.4	12.5	9.1
6	8.3	1.9	11.2	4.8	17.9	11.5
12	12.4	0.0	16.7	4.3	21.3	8.9

$\Delta\pi$ = Difference between the osmotic potential of the sap and the osmotic potential of the growth solution (0.4 atm)

Although the exact mechanism of growth inhibition was not pinpointed, Bernstein (4) drew the following conclusions:

> ... Water-absorbing capacity appears to be relatively unaffected by salinity. At least transpiration per unit leaf area and water requirement may not be particularly affected. Total transpiration per plant or culture does, of course, decrease markedly with increasing salinity but this is the result of sharply inhibited growth and large **decreases** in leaf area. ... Turgor is maintained at the same level as in nonsaline cultures by increases in OP. ... However to maintain this turgor (and leaves that are still expanding apparently do maintain it) continuous cellular accumulation of solutes to the level of the higher OP is required. This is the proposed growth limiting process.

Gauch and Wadleigh in 1944 (20) found that iso-osmotic concentrations of sodium chloride, calcium chloride and sodium sulfate, added to a complete nutrient solution caused nearly equal growth inhibition of red kidney beans. (See Figure 2) Plants grown in nutrient solutions with additions of magnesium chloride or magnesium sulfate showed much greater growth inhibition. To eliminate specific ion effects Ruf et al (59) used Carbowax 1540 (polyethylene glycol) to raise the osmotic potential of the growth solution without introducing sodium or chloride ions. They found that growth of wheatgrass and potato was inhibited in proportion to the osmotic potential of the growth solution. Ruf et al (59) also found that the saps of the plants adjusted their osmotic potential slightly more than 80 percent of the amount the nutrient solution osmotic potential was increased (74).

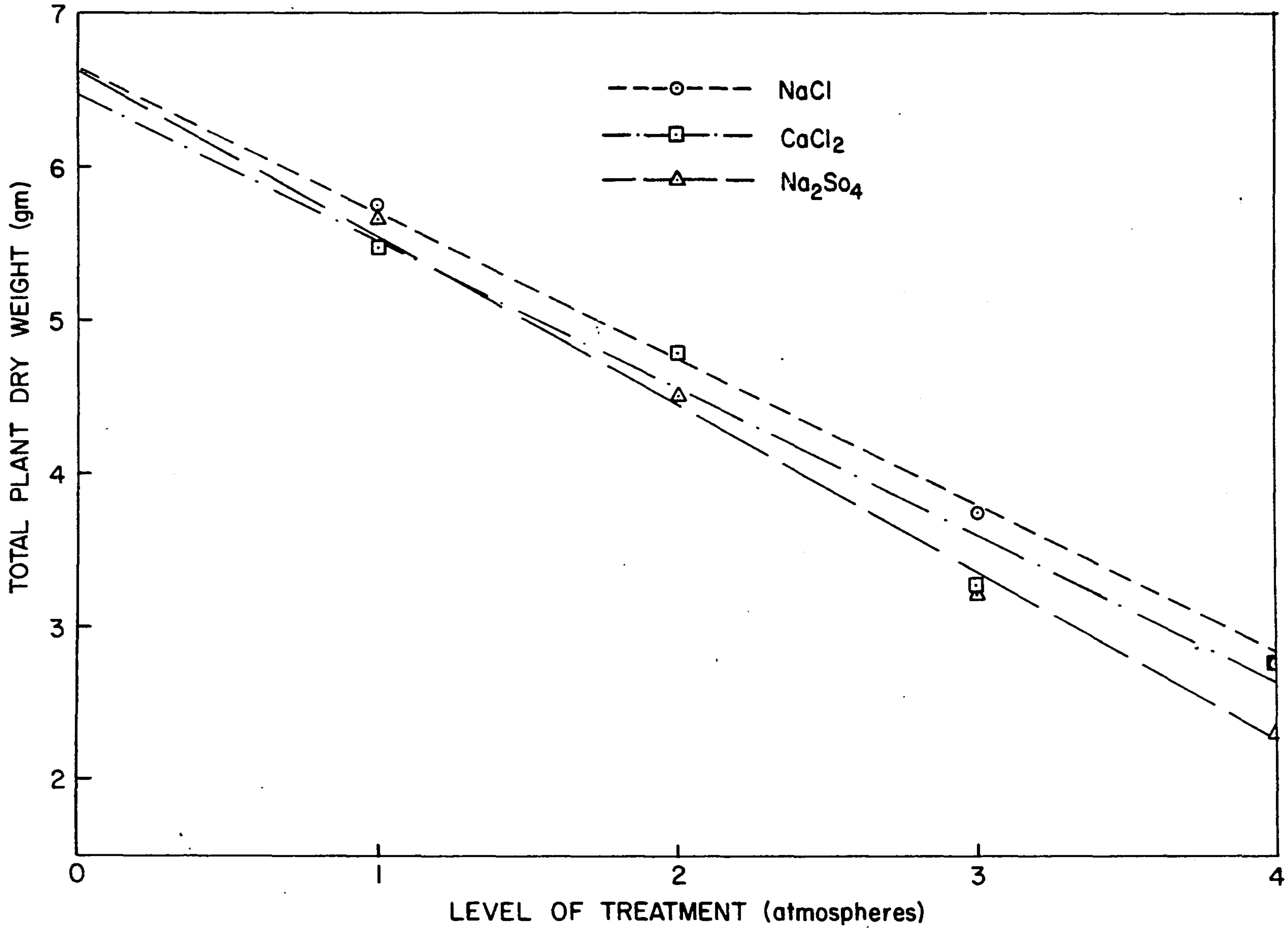

Fig. 2.--Iso-osmotic Growth Inhibition of Red Kidney Beans (Data from Gauch and Wadleigh (20))

Wadleigh and Ayers (74) subjected red kidney beans to numerous combinations of water and osmotic stress. Their results, shown in Figure 3, indicate that growth inhibition is proportional to the total stress experienced by the plant whether it is generated by sodium chloride or soil moisture stress or a combination of both.

1.32 Specific Ion Effects. Harris et al in 1924 (21) found that native plants able to grow under saline conditions (halophytes) possessed a high sap osmotic potential. In addition, halophytes also possess the ability to accumulate potassium in preference to sodium even when the concentration of sodium in the soil is much greater than the concentration of potassium. Typical ratios of potassium to sodium in the sap of marine halophytes are shown in Table III, Page 14. Non-halophytic plants growing in non-saline soils also have the ability to accumulate potassium in concentrations greater than it occurs in the soil. However, the latter lose this ability as the concentration of sodium in the soil or in the growth solution is increased. The potassium to sodium ratio in the sap of pepper plants treated with various levels of sodium chloride is shown in Table IV, Page 14. The apparent competitive inhibition of potassium accumulation by sodium has led to much speculation about sodium-potassium uptake mechanisms (15), (16), (71) and is responsible for the postulate that both ions are bound to the same site on the root (27).

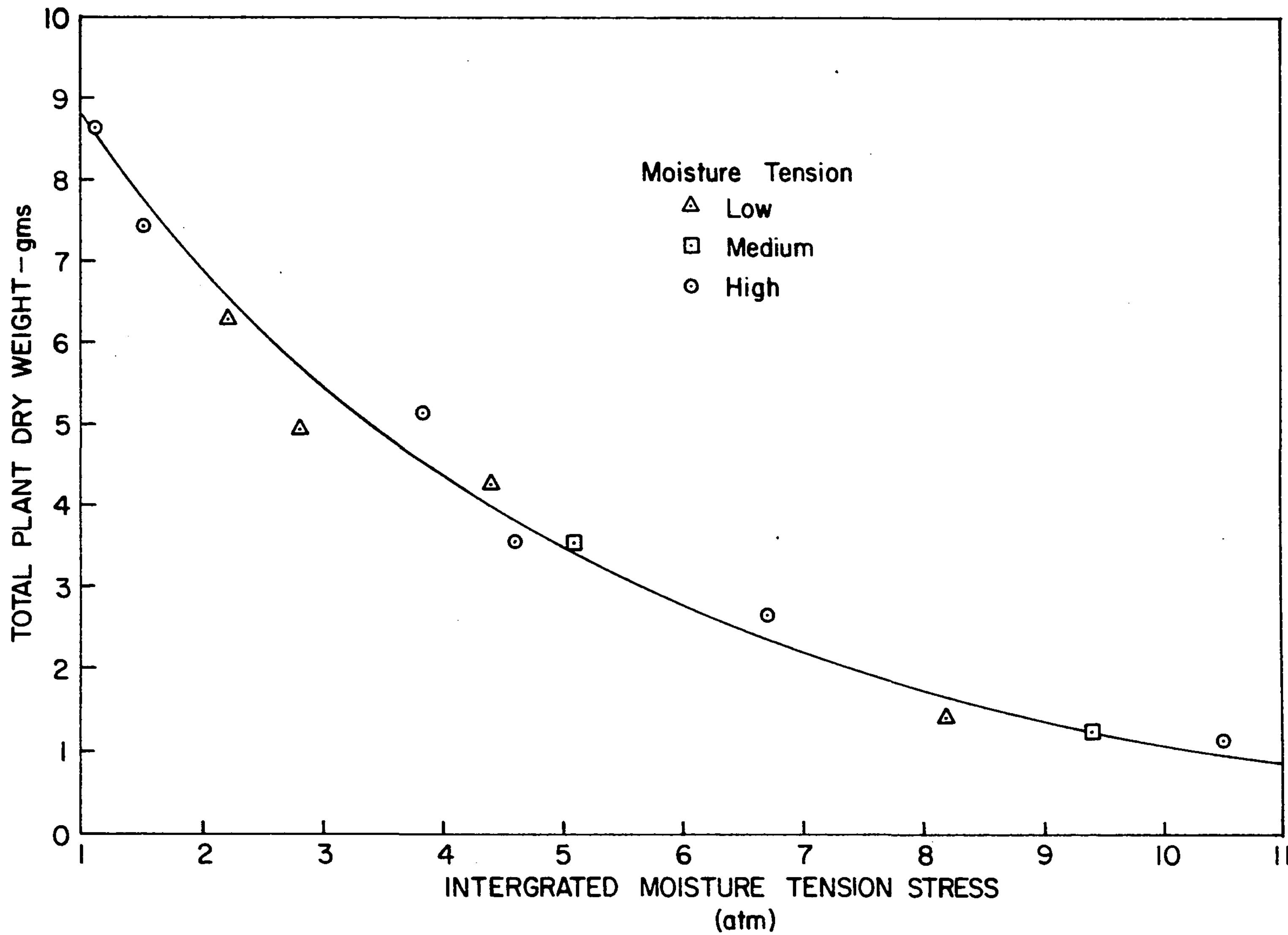

Fig. 3.--Relation of Red Kidney Bean Growth to the Integrated Moisture Stress (Data from Wadleigh and Ayers (74))

TABLE III

POTASSIUM TO SODIUM RATIO IN THE SAP FROM MARINE HALOPHYTES (65)

Plant Type	K^+/Na^+
Rhodymenia palmata	78.0
Ulva lactuca	10.4
Lammaria saccharina	6.4
Pelvetia canaliculata	1.5
Sea Water (for comparison)	0.04

TABLE IV

CHANGE IN THE POTASSIUM TO SODIUM RATIO IN SAP FROM RINSED PEPPER ROOTS TREATED WITH SODIUM CHLORIDE (4)

Osmotic Addition of NaCl (atm)	K^+/Na^+
0	12.70
1	2.00
2	0.93
3	0.62
4	0.58

Even plants which adjust the osmotic potential of their sap display certain specific ion relationships. For example, the roots of red kidney beans making an osmotic adjustment to additions of sodium chloride to the growth solution showed a decrease in the potassium concentration of the root sap. (See Figure 4) Competitive inhibition might explain this result but Bonner and Galston (7) offer another explanation:

> It is impossible, of course, for a plant simply to take up ions of one charge and leave ions of the opposite charge in the outside solution, since large electrical fields would be set up. Electrical balance of anions and cations must be preserved both inside and outside the cell. An excess of cation uptake must be accompanied by changes in ionic composition of the cell and nutrient as to maintain electrical neutrality in both places.

The decrease in potassium with the increase in sodium might be involved in maintaining electrical neutrality.

The trading of one monovalent ion for another to maintain electrical neutrality would not in itself enable a plant to make an osmotic adjustment. However, electrical neutrality can be maintained during osmotic adjustment if the plant exchanges one divalent cation for two monovalent cations in the growth solution. For example, the plant might accumulate sodium or potassium while rejecting calcium. Such an exchange would double the number of cations in the

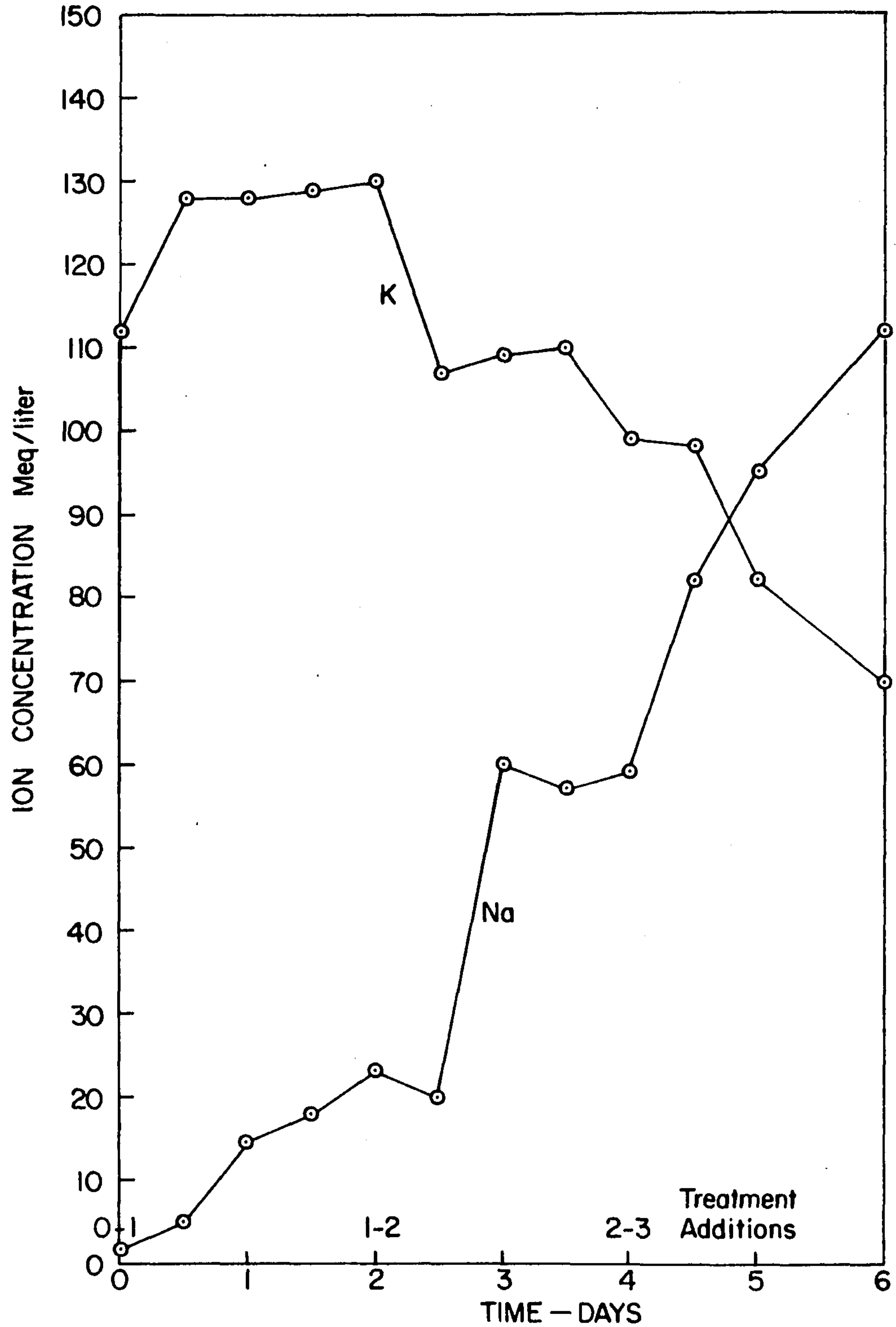

Fig. 4.--The Concentration of Sodium and Potassium in the Root Sap of Red Kidney Beans Experiencing Increasing Levels of Sodium Chloride (Data from Bernstein (5))

plant and thereby increase the osmotic concentration. Similarly, substitution of chloride anions for polyvalent organic acids in the plant would also increase the internal osmotic potential while maintaining electrical neutrality (4). Ulrich in 1941, (69), showed that organic acid content of cells decreased when anions (such as chloride) were accumulated in excess of cations. He found the reverse also to be true--that is, the organic acid content of the cells increased when cations were accumulated in excess of anions. His results indicated that organic acid synthesis actually preceded the excess accumulation of cations.

Hendricks in 1964, (24), noted that when salt solutions of different concentrations are on two sides of a membrane, an electrical potential is established in the pores where the two solutions are in contact. The controlling factor in the selectivity of membranes for one cation over another is the amount of electrostatic attraction between the cation and the negatively charged wall of the membrane pore. Observations by Etherton and Higinbotham in 1960 (17) indicated that the root solution in an intact root normally has a negative electrical potential approximately 100 millivolts below that of the external solution. The potential difference between the root and the growth solution is decreased if the concentration of the external solution is increased. Bernstein (5) reported a study by Dainty in 1959 which indicated that the potassium content of algae cells is dependent upon the electro-chemical potential of the cell contents. Accumulation of other cations causes the electrical gradient between the growth solution and the cell to decrease which results in the loss of the potassium from the cell to the solution.

1.4 Salinity Toxicity Symptoms

Although no case has been established for specific chloride or sodium toxicity to any crop (2) the symptoms of plants growing under high salinity conditions with high concentrations of one or more ions have been recorded. A comparison of the symptoms of salinity toxicity, potassium deficiency, and drought is shown in Table V.

In addition to producing symptoms resembling drought and potassium deficiency, several instances of salinity interference with nitrogen metabolism have been reported. Strogonov (64) noted a higher population of male trees on saline soils and an increase in the ratio of male flowers to female flowers on plants growing in saline areas. It has been reported also that a nitrogen deficiency causes the ratio of male flowers to female flowers to increase (30). In addition,

TABLE V

COMPARISON OF SYMPTOMS OF SALINITY TOXICITY TO THOSE OF POTASSIUM DEFICIENCY AND DROUGHT

Salinity Toxicity Symptoms	Potassium Deficiency Symptoms	Drought Symptoms
General	General	General
Reduction in size of leaves, height of plant, diameter of stems, and yield (23).	Plant's growth stunted, internodes short, yield decreased. (75)	Stunted growth and yield. (74)
Foliar	Foliar	Foliar
Sodium Chloride - light green to yellow primary leaves. Young trifoliate leaves--dark green (20).	Leaves may be dull, bluish-green with marginal scorching, tip burn and development of brown spots, usually more numerous at the margins (75).	Stomatal closure, desiccation of older leaves. Younger leaves may remain turgid by translocation of water from older leaves and fruit (33).
Calcium Chloride - yellow, green primary and trifoliate leaves. Second trifoliate and younger trifoliate yellow-green to green (20).		
Sodium Sulfate - margin and tip burn--brown necrotic tips and margins of primary leaves. Young trifoliate leaves dark green (20).		
Sodium - necrotic or scorched spots near the margin or the interior of the leaf (23).		
Chloride - necrotic burning at the tip of leaf progressing down the blade and sometimes along the margins. Leaves tend to be thicker and more succulent (23).		

Nightingale and Farnham (50) reported the following in 1936:

> In. . . many of our perennial plants. . . the initial stages of protein synthesis occur almost exclusively in the fine succulent rootlets; but in a solution high in concentration of salts, roots rapidly become woody, optically empty, and lose their capacity for protein synthesis. Nitrate absorption is not limited but the roots lack the capacity to assimilate nitrate, that is, synthesize amino acids and proteins. The effect will therefore be that of "nitrogen deficiency" or more accurately that of protein deficiency.

This seems to fit the results of El Shourbagy (14) who found the growth inhibition by sodium chloride, of tomato roots grown *in vitro*, could, in some instances, be partially compensated for by the addition of a mixture of amino acids.[1]

1.5 Summary of Salinity Research (See Figure 5)

There appears to be no doubt that high levels of salinity cause a decrease in yield. To date no conclusive evidence of specific toxicity to any vital metabolic process has been found for sodium or chloride. Rather, growth inhibition appears to be related to the high osmotic potential of the water surrounding the roots growing under saline conditions. The symptoms of plants exposed to salinity resemble the symptoms of plants experiencing drought. However, studies have shown that simple physiological drought is probably avoided in plants treated with moderate

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[1] Mixture contained following amino acids at 10^{-7}M concentration: threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, cystine, valine, and lysine-HCl.

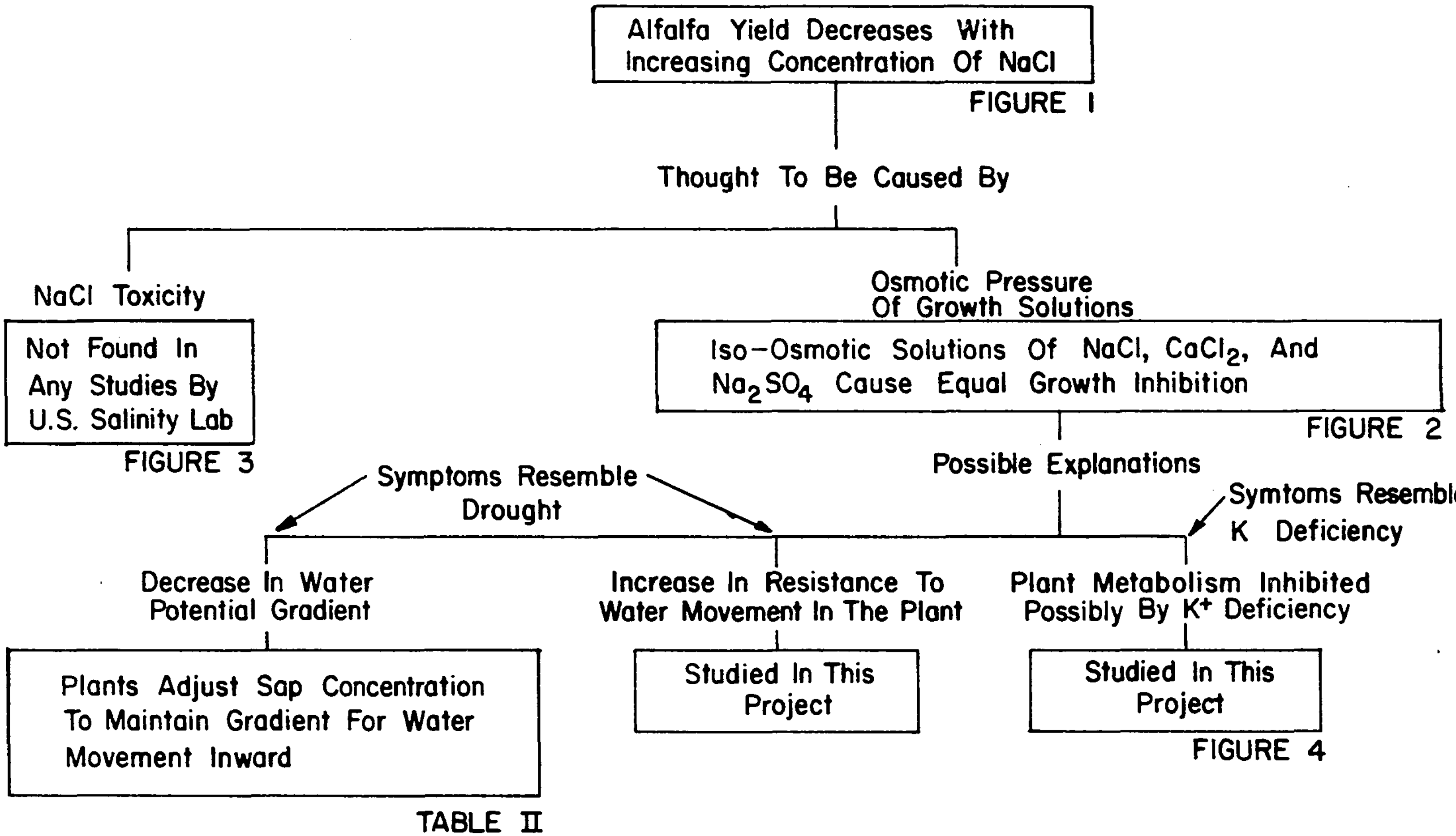


Fig. 5.--Summary of Salinity Research Prior to Project Initiation

levels of salinity, by osmotic adjustment of their sap to maintain an inward gradient for water movement. Drought symptoms might still occur if the resistance of the water conducting tissue were increased under saline conditions. Iso-osmotic growth inhibition does not per se preclude the consideration of specific ion effects. Increasing the osmotic concentration of the growth solution may produce a disruption of the normal metabolic processes such that potassium is lost from the roots or the rate of nitrogen assimilation into proteins and amino acids is decreased.

CHAPTER 2

EXPERIMENTAL DESIGN AND PROCEDURES

2.1 Objectives

This experiment was designed to detect, identify, and explain the physiological changes that occur in plants irrigated with moderate levels of saline water. Special emphasis was given to changes in water potentials, tissue resistance, and ion accumulation rates. Observations were made to verify the results of previous studies showing iso-osmotic growth inhibition and osmotic adjustment of sap under saline conditions.

2.2 Design

2.21 Plant Selection. Red kidney beans (*Phaseolus vulgaris* L.) were selected for this study since previous investigators of salinity, Gauch and Wadleigh (20), Bernstein (4), (5), and Largerwerff and Eagle (39), have used these plants extensively. In addition, red kidney beans are relatively salt sensitive; thus, their response to salinity is more pronounced than for relatively salt tolerant plants (6).

2.22 Treatment Selection. Treatments of sodium chloride, calcium chloride, and sodium sulfate were primarily chosen because of the previous work by Gauch and Wadleigh (20) showing that at iso-osmotic concentrations these salts gave equal growth inhibition. However, it is interesting to note that 55% of the aquifers in the U. S. containing 1000 ppm or more of dissolved solids contain as their major constituent either sodium chloride, calcium chloride, or sodium sulfate (18). The most frequent contaminating salt is sodium chloride. Since one of the objects of the study was to separate specific ion effects from osmotic effects, Carbowax 1540 was used as the osmotic agent in some of the experiments.

Concentrates of the salts were prepared and metered out into bottles containing sufficient solution to raise the osmotic pressure of the growth solution by one atmosphere. The increments were not all equal, thereby making it necessary to prepare separate bottles for each osmotic step for each salt. The amount of salt required for each osmotic step was calculated as follows: The data in the Handbook of Chemistry and Physics (26) relating concentration to freezing point depression were used to find the freezing point depression of various concentrations of each salt. The values of the freezing point depressions were substituded into Equation 2. 1 given by Bernstein (4):

$$\pi = 12.06\Delta - 0.021\,\Delta^2 \qquad 2.1$$

where π = osmotic potential (atm.)

Δ = freezing point depression (^{o}C)

From these data a graph was constructed relating concentration to osmotic potential. From this graph the concentration changes required for each osmotic increment were determined. These data are tabulated in the Appendix.

In order to check the validity of using the relationships of pure salt solutions for calculating salinity additions, the conductivity and osmotic potential of nutrient solutions with the various salts added were measured. These observations showed the relationship between osmotic potential and conductivity for salts added to nutrient solutions is nearly the same as the corresponding relationship for pure salt solutions. Thus the data for pure salt solutions were used to calculate the salinity additions.

Carbowax 1540 was purchased from the Union Carbide Corporation in 50-pound drums. It has a relatively low melting point (38-40C) (46), but at room temperature it is a solid, closely resembling paraffin. In contrast to paraffin, which is insoluble, Carbowax 1540 is extremely soluble in water. One-half bar osmotic increments were prepared by pouring the prescribed amount of melted Carbowax into paper drinking cups (without coating of paraffin). The Carbowax in the cups solidified on cooling. The increments, so prepared, were then stored until needed. Similar to the salt additions, the half-bar increments are not equal, so it is necessary to prepare increments for each half-bar separately. The data used to calculate the osmotic additions of Carbowax 1540 were provided by Nichols (45) and are shown in the Appendix.

2.23 Treatment Levels and Application. The additions of all chemicals, designed to raise the osmotic potential of the growth solutions, were made in the evenings after sunset so that the initial phase of osmotic adjustment could occur during a period of low transpiration. High rates of transpiration during this initial period would cause severe wilting and tissue damage. According to the procedure utilized by Bernstein (5), the salt levels were increased one atmosphere every 48 hours until they reached their final levels. The Carbowax treatments were increased in one-half bar increments every 24 hours since previous investigators have found more rapid increases cause blocking of the vascular system (45).

Initially it was planned to have osmotic additions to the nutrient solution of 0, +1, +2, +3, and +4 atmospheres for each treatment. Because of space and personnel limitations the five levels of treatment would have permitted only two plants from each treatment to be harvested at each harvest. It was decided that it was more important to have more replications per harvest so the treatments were reduced to 0, +2, and +4 atmospheres. The total concentration of added salts in each treatment is shown in Table VI, Part (a). For comparison, the concentration of dissolved solids in several natural waters also is given in Part (b) of Table VI.

2.24 Nutrient Solution. The nutrient solution used was one previously used by O'Leary (51) and is a modification of a nutrient solution prepared by Meyer and Swanson (44). The ingredients, their concentration and the resulting concentration of ions is shown in the

TABLE VI

CONCENTRATION OF DISSOLVED SOLIDS

(a) Treatments Added to Growth Solutions (ppm)

Treatment	Level	
	+2 atm	+4 atm
NaCl	2,835	5,741
$CaCl_2$	3,775	7,638
Na_2SO_4	5,157	10,783

(b) Natural Waters (ppm)

Source	Observation	Concentration
(31)	Colorado River, Imperial Dam	917
(31)	Colorado River Morelos Dam	2,730
(63)	Cienega Creek	876
(32)	Standard sea water	34,330
(32)	Red Sea	40,000

Appendix. Deionized, distilled water was used for the preparation of the nutrient concentrates and in the final solution in which the plants were grown.

The plants were grown in plastic wastebaskets (Rubbermaid No. 2940) coated with two layers of black and one layer of white paint to keep light out of the nutrient solution. Preliminary experiments with these containers unpainted showed that vigorous algae growth developed in the nutrient solutions. A square wooden plywood lid was cut to fit over the top of each container. The lids had slots so the plants could be removed intact at harvest time. A piece of butyl rubber stapled to the lid served to cover the slot and other access holes when they were not in use. The covering of the solution is necessary to prevent light entry and to decrease evaporation from the solution. The solutions were aerated by compressed air through aquarium type aeration stones.

2.25 Environment. The experimental plants were grown in a conventional greenhouse. The house was glazed with one layer of polyethylene plastic (GER-PAK 601). A saran shade was placed over the plant growth area during the experimental period. The inside solar radiation was approximately thirty percent of the outside insolation. The temperature in the house was controlled by evaporative cooling. The north wall of the greenhouse was composed of aspen fiber cooler pads. Air was pulled through the pads by two exhaust fans mounted on the south wall. During the periods of intense solar heating, water was passed over the cooler pads and the incoming air was evaporatively cooled. During the experimental period the temperature usually ranged from a maximum of 30°C to a minimum of 21°C. The relative humidity normally reached a minimum of 60% in the daytime and approached saturation at night.

2.26 Germination and Treatment Schedule. On July 18th, 1966, 800 bean seeds were planted in a vermiculite seedbed. The seeds were watered with tap water during their stay in the seedbed. A heavy saran shade was placed over the seedbed until July 25th, at which time the 30% saran shade was placed over the entire growing area of the greenhouse. Three hundred of the seedlings, each with two healthy primary leaves, were transferred to the containers with nutrient solution on the evening of July 27th. Previous studies at the Environmental Research Laboratory indicated that red kidney beans with less than 13 days in the seedbed developed no fruit after 35 days growth in nutrient solution (56). The development of fruit causes changes in the ion distribution in the plant and greatly complicates the study of osmotic adjustment (65). On the evening of August 3rd, eight days after planting, (and continuing to the morning of August 4th) the nutrient solutions of all the plants were changed. On August 4th, twelve plants were removed from containers near the ends and center of the growing area. These containers without plants were used to estimate the evaporation from the surface of the nutrient solutions.

The osmotic treatments were initiated for Carbowax on the evening of August 5th and for the salts on the following evening. This procedure permitted the plants in all treatments to reach their final nominal treatment levels at the same time. The +2 atm treatments reached their final level of treatment on August 8th and the +4 atm treatments attained their final level of treatment on August 12th.

2.27 Harvesting Procedures. The experimental design called for twelve harvest periods. All the plants in one harvest were grouped together on the growing tables. The position of the harvest groups was randomly distributed throughout the growing space. Within a harvest group the treatments were randomly distributed. This arrangement permitted the treated plants and the control plants, to which the treated plants would be compared, to experience the same growth environment. The random position of the harvest groups was designed to eliminate bias due to spatial differences in environment.

Three harvest groups, 2, 11, and 13, containing three plants per treatment (including the control), were scheduled to determine the physical characteristics of the plants. The remaining harvests were primarily designated for sap analysis. Four plants from each treatment were harvested in each of these harvests. A complete harvest schedule and treatment schedule are shown in Table VII.

TABLE VII

TREATMENT AND HARVEST SCHEDULE

Seeds planted in seedbed 7/18/66

Date	Time	Total No. of Plants Harvested: Physical Characteristics	Total No. of Plants Harvested: Sap Analysis	Base Nutrient 0	Treatment Levels - Atmospheres*: +1	+2	+3	+4
8/5	AM							
	PM		4	H(4)**	0—1/2			
8/6	AM	3		H(3)	1/2—1			
	PM		4	H(4)	0—1			
8/7	AM		20	H(4)	H(4)			
	PM					1—1-1/2		
8/8	AM					1-1/2—2		
	PM		16		H(4)	1 — 2		
8/9	AM		36	H(4)	H(4)	H(4)		
	PM						2—2-1/2	
8/10	AM						2-1/2—3	
	PM		16			H(4)	2 - 3	
8/11	AM		36	H(4)		H(4)	H(4)	
	PM							3—3-1/2
8/12	AM							3-1/2—4
	PM		16				H(4)	3 - 4

(Table Continued on Next Page)

TABLE VII--Continued

Date	Time	Total No. of Plants Harvested: Physical Characteristics	Total No. of Plants Harvested: Sap Analysis	Base Nutrient 0	Treatment Levels - Atmospheres* +1	+2	+3	+4
8/13	AM		36	H(4)**			H(4)	H(4)
	PM							
8/14	AM	27		H(3)		H(3)		H(3)
8/15	AM		11	H(3)				H(2)
8/16	AM	— Nutrient change						
8/17	AM							
8/18	AM							
8/19	AM							
8/20	AM							
8/21	AM		36	H(4)		H(4)		H(4)
8/22	AM	27		H(3)		H(3)		H(3)

* One atmosphere **steps** are for salts and 1/2 atm **steps** are for Carbowax.
** Number of plants harvested per treatment.

2.3 Data Collection Procedures

2.31 Physical Characteristics Harvests. Three replicas of each treatment were harvested for each physical characteristics harvest. The plants were harvested between 6 a.m. and 8 a.m. MST on the designated days. Immediately after their removal from the growth solution, the plant's roots were wrapped in a paper towel, then the entire plant was inserted into a plastic bag. A sample of the nutrient solution was taken after each plant was removed. After all the plants had been harvested, the plants were cut, one by one, into leaves, stems plus petioles, and roots. The fresh weight of each portion was recorded. The roots were then placed in paper bags for insertion into an oven and dry weight determination. Blueprint copies of the leaves and stems with petioles were made before these tissues were placed in bags for drying.

The petioles of the youngest leaves in the Harvest 13 plants were tagged on 8/11 and 8/15 with split sections of plastic drinking straws. When these plants were harvested, the leaves were separated into their periods of initiation according to whether they developed before or after the tags were applied.

2.32 Sap Analysis Harvests. Four plants from each treatment were harvested on dates designated for sap analysis harvests. The morning harvests were conducted between the hours of 6 and 8 a.m. MST and the evening harvests between the hours of 6 and 8 p.m. MST.

The harvesting technique, including the collection of the nutrient sample, was the same as for the physical characteristics harvest.

The fresh weight of the roots, stem plus petioles, and leaves was recorded for two of the plants. Then ten grams of tissue from each of the portions of the plants were wrapped in aluminum foil for later sap expression with the Carver Press. The remaining plant tissue was discarded. When all the samples had been collected, the packets of aluminum foil containing tissue were submerged in liquid nitrogen and then stored at -5°C until the time when their sap was to be expressed. At this time they were thawed and inserted in the chamber of the Carver Press. The sap sample obtained was then stored at 5°C. Only ten grams of sample were removed for sap expression since previous studies indicated that the composition of the sap was a function of the sample mass (9).

The fresh weight of the leaves, roots, and stems with petioles, of the other two plants harvested in these harvests was also recorded. The leaves and stems with petioles were put in bags for drying in the oven. Ten grams of the root tissue were rinsed in deionized, distilled water and prepared for sap analysis as indicated above. The remainder of the root tissue was usually discarded. However, on 8/21, Harvest 12, a portion of the root tips was removed from each treatment and preserved in the standard manner in a mixture of formalin, acetic acid, and alcohol (FAA) (10).

2.33 Plant Growth and Metabolism Observations. On four occasions silicone rubber impressions were made of leaves from all the treatments according to the technique developed by Sampson (60). After each impression, a blueprint copy of the leaf was made. The silicone rubber negatives were stored in small envelopes until they were to be analyzed.

On two occasions root tips and leaf discs were removed from control and treated plants and inserted into Warburg flasks. The rate of photosynthesis of the leaves and the rate of respiration of the roots were determined by the standard procedure (70), (36).

2.34 Water Metabolism Observations. The daily evapotranspiration for each plant was determined by metering the water required to bring the nutrient solution up to the same level each day. Deionized distilled water was added to each container from a 500 ml buret. A water level indicator was inserted into the hole provided for it in each container lid. When the nutrient solution reached the reference level it made contact with the tip of a metal probe which then lit a small light bulb. The amount of water added to the nearby containers without plants was assumed to equal the evaporation rate from the containers with plants. The daily evaporation was subtracted from the daily evapotranspiration to calculate the daily transpiration.

The resistance the leaves offered to vapor diffusion was measured with a leaf resistance meter on five occasions. Several modifications were made on the meter described by vanBavel et al (73). The modifications are described in an internal publication of the Arizona Water Resources Research Center by Oliveira (53). In this method the decrease in rate of vapor transfer, to a lithium chloride cell held close to the leaf, below that observed over a saturated surface is attributed to the resistance the stomates and cuticle offer to vapor movement.

The resistance the roots offered to water movement also was measured. Pressure was applied to the water in a chamber in which detopped plant roots were placed. The pressure chamber was essentially the same as that used by O'Leary (52). The rate of exudation was measured over a period of several hours and the resistance was calculated as the ratio of root fresh weight to the flow rate.

The total leaf water potential, ψ, was measured on five days throughout the experimental period, utilizing the Shardakov dye technique as described by Kramer and Brix (34). A summary of the data collection periods is shown in Table VIII.

2.4 Methods of Data Analysis

The blueprints of the leaves were planimetered to determine their area. The area per leaf was then summed over the entire plant to determine the total leaf area per plant. The stomatal density was calculated by making a nail polish positive from the silicon rubber impression of the leaf surface. The clear positive was then viewed

TABLE VIII

SUMMARY OF DATA COLLECTION

Date	Transpiration	Nutrient Sample	Plants Tagged	Leaf Impressions	P.S. and Respiration	Leaf Water Potential	Leaf Resistance	Root Resistance
8/5	x	x						
8/6	x	x						
8/7	x	x				x	x	
8/8	x	x						
8/9	x	x				x		
8/10	x	x	x	x				
8/11	x	x				x	x	
8/12	x	x		x				
8/13	x	x				x		x
8/14	x	x		x	x			
8/15	x	x	x				x	
8/16	x	x						
8/17	x	x						
8/18	x	x					x	
8/19	x	x						
8/20	x	x					x	
8/21	x	x				x		x
8/22	x	x		x	x			

under a microscope with a grid in the ocular and the number of stomates in the field was counted. Five fields were viewed on each impression.

The concentration of the cations of sodium, potassium, magnesium, and calcium in the nutrient solution samples was determined by analysis on the Perkin-Elmer Atomic Absorption Spectrophotometer (Model 303). The chloride content of the nutrient solutions was measured by titration with silver nitrate using the procedures described in Diagnosis and Improvement of Saline and Alkaline Soils (55). The osmotic potential of the nutrient solution was measured with an Advanced Osmometer. Unfortunately, the osmotic potential of sap samples could not be analyzed directly with the osmometer since natural particles were acting as ice-nuclei and preventing the required sub-cooling of the sample. Studies by Fukuta (19), Parungo (54), and others have shown that many organic species possess ice nucleating powers; thus, it is difficult to say which constituent was causing premature freezing. However, a good correlation was found between the conductivity of the nutrient solutions and the osmotic potential for each of the treatments and these relationships were used to estimate the osmotic potential of the saps of plants growing in the corresponding solutions. The regression equations relating conductivity to osmotic potential are shown in the Appendix. The electrical conductivity of the saps and of the nutrient solutions was measured with a conductivity bridge. (Industrial Instruments Model RC 16 B2)

Since the treatment salts were taken up in large quantities by the plants, the conductivity of the plant sap was related to osmotic potential in approximately the same manner as the nutrient solution. Bernstein (4) found that the ratio of the osmotic potential to the electrical conductivity in plants treated with sodium chloride was 0. 39 atm/millimho between 2 and 4 atm. The same ratio for a pure salt solution of sodium chloride is 0. 38 atm/millimho.

A more detailed description of greenhouse and laboratory procedures can be found in Internal Report FY 67-3 (76) of the Arizona Water Resources Research Center.

CHAPTER 3

RESULTS

3.1 Plant Growth and Metabolism

3.11 Total Plant Dry Weight. The results presented in this section and in the remainder of this chapter were analyzed for their statistical significance where sufficient data were collected. The t-test (41) was used to detect significant differences in the mean of the control plants and each of the means of the treated plants. Where significance existed it is noted in the table by one or two asterisks, depending on the level of significance. Significance in this case means that the difference between the mean of the control plants and the mean of the treated plants is probably real, with the level of significance indicating the probability, expressed as a percent, that this judgment is in error. In addition, an analysis of variance (41) of the treatments only was carried out to determine the magnitude of the variance between levels of treatment (columns) and between the different treatments (rows). The results of the analysis of variance were evaluated with the F-test (41) and are noted in the right margin of each table. The difference between the Carbowax treatment means at each level and the means of each of the salt treatments were compared using as a measure of significant differences the least significant difference, (l.s.d.), (41).

The same measure was used to compare the two levels of each treatment. Where differences between means exceed the l. s. d., the fact is noted in the text.

Three plants were harvested from each treatment on 8/6, prior to the application of treatments; on 8/14, after all the treated plants had been brought up to their nominal levels of treatment; and on 8/22, after the plants had been growing at the nominal treatment levels for more than one week. The average of the total plant dry weight for each treatment on these dates is shown in Table IX. Although growth inhibition was evident on 8/14, it is not statistically significant. However, the trend had already been established by this time in that the control plants had a greater dry weight than the plants treated with Carbowax, and the Carbowax treated plants had dry weights exceeding any of the salt treated plants. This difference was not statistically significant on 8/14, but on 8/22 the mean of both the sodium chloride and calcium chloride treatments at the 2 atm level were less than the Carbowax mean at the same level by an amount exceeding the l. s. d. Another general characteristic that had developed by 8/22 was that the growth decreased with the increased level of treatment for all the chemicals added to the nutrient solution. The decrease with increasing level of treatment was slight for sodium chloride and calcium chloride treated plants, but the decrease in total dry weight between 2 atm and 4 atm for plants treated with sodium sulfate and Carbowax was greater than the l. s. d.

TABLE IX

MEAN PLANT DRY WEIGHT

grams

Date	Treatment	+2 atm Level			Mean	+4 atm Level			Mean	Analysis of Variance Results (Treatments Only)
8/6/66	Control	4.10	2.92	3.32	3.45	4.10	2.92	3.32	3.95	
	Control	15.54	20.19	10.90	15.54	15.54	20.19	10.90	15.54	Analysis of variance
	NaCl	10.32	13.11	9.03	10.82	14.51	14.31	6.47	11.76	not done since "t"
8/14/66	$CaCl_2$	11.75	10.30	8.99	10.35	8.27	11.15	16.73	12.05	test showed no signi-
	Na_2SO_4	12.52	11.53	9.73	11.26	14.44	11.58	6.39	11.80	ficant difference be-
	CBWX	14.51	9.81	10.93	11.75	10.93	14.96	11.58	12.49	tween control and treated.
	Control	33.98	48.31	36.56	39.62	33.98	48.31	36.56	39.62	Variance between
	NaCl	22.14	23.56	22.87	22.86**	23.51	16.58	23.99e	21.36*	rows significant at
8/22/66	$CaCl_2$	22.13	18.00	28.36	22.83*	20.31	15.29	20.78	18.79**	5% level. Variance
	Na_2SO_4	30.27	31.33	30.80	30.80	16.38	15.02	23.46	18.29**	between columns
	CBWX	30.86	36.25	33.76	33.62	28.37	14.63	21.84	21.61*	significant at 1% level. F-Test

e - Dry weight of stem estimated from fresh weight using ratio of fresh weight to dry weight observed in other two replicates.

* 5%) Significance One Tail "t" test
** 1%)

Although the plants did not respond equally at the intermediate level of treatment, the growth inhibition caused by the 4 atm treatments was nearly equal for all treatments and in no case did the difference between means exceed the l. s. d.

Since most of the significant changes in total plant growth were not evident until 8/22, the next sections will look in more detail at the data from plants harvested on this date.

3.12 Leaf Growth and Metabolism

3.121 Physical Characteristics. Leaf dry weight and water content data are shown in Table X. The average dry weight of the leaves of plants treated with Carbowax is less than the dry weight of the leaves from the control plants. The salt treated plants' leaf dry weight is less than the Carbowax plants' leaf dry weight but not by a statistically significant amount. The suppression of leaf tissue production, as measured by leaf dry weight, increases with the level of treatment, but not by a statistically significant amount.

The decrease in the water content is the most statistically significant suppression of growth recorded for the leaves. At the intermediate level of treatments, the water content of the leaves of the Carbowax treated plants is more than that in the salt treated plants, but not by a statistically significant amount. The leaf water content is decreased significantly as the level of treatment increases for both the Carbowax and sodium sulfate treatments. The decrease in the Carbowax plants'

TABLE X

PHYSICAL CHARACTERISTICS OF LEAVES
8/22 Data

Variable	Treatment	+2 atm Level				+4 atm Level				Analysis of Variance Results (Treatments Only)
					Mean				Mean	
Dry Weight/ Plant gm	Control	15.76	23.29	17.29	18.78	15.76	23.29	17.29	18.78	No significant variance. F-Test
	NaCl	11.13	11.17	---	11.15	11.79	11.12	5.91	9.61*	
	$CaCl_2$	11.41	8.70	13.50	11.21*	9.59	8.17	9.90	9.22*	
	Na_2SO_4	14.65	14.60	---	14.63	8.50	7.04	13.57	9.71**	
	CBWX	14.88	17.45	15.55	15.96	12.52	7.49	10.29	10.10*	
Water Content/ Plant ml	Control	115.93	140.98	134.53	130.47	115.93	140.98	134.53	130.47	Variance between columns significant at 1% level. F-Test
	NaCl	70.63	93.69	---	82.16*	75.19	93.74	81.66	83.52**	
	$CaCl_2$	92.87	62.49	110.08	88.47*	65.48	60.93	71.09	65.83**	
	Na_2SO_4	86.71	83.83	---	85.27**	54.68	50.12	54.41	53.06**	
	CBWX	83.39	98.28	93.32	91.65**	67.58	32.38	53.09	51.01**	
Water/Dry Weight ml/gm	Control	7.36	6.05	7.78	7.06	7.36	6.05	7.78	7.06	Variance between rows significant at 5% level. F-Test
	NaCl	6.35	8.39	---	7.37	6.38	8.43	13.82	9.54	
	$CaCl_2$	8.14	7.18	8.15	7.82	6.83	7.46	7.18	7.16	
	Na_2SO_4	5.92	5.74	---	5.83	6.43	7.12	4.01	5.85	
	CBWX	5.60	5.63	6.00	5.74*	5.32	4.32	5.16	4.93*	

* 5%)
** 1%) Level of significance One Tail "t" test

leaf water content was greater than in the salt treated plants. At 4 atm the Carbowax plants had a leaf water content nearly equal to the water content of the sodium sulfate leaves but lower than either of the chloride treated plants. The difference between the mean leaf water content of the Carbowax plants and the sodium chloride plants at the 4 atm level is greater than the l. s. d.

Since the leaf water content and the dry weight are decreased by the treatments, the question then arises, are both these variables decreased in the same proportion? The ratio of the leaf water content to the leaf dry weight answers this question. In addition, this ratio gives us a measure of the succulence of the leaves. This is an important variable to consider since it gives us some insight into the degree of tissue desiccation.

The data show that plants treated with sodium sulfate or Carbowax have a lower leaf water content per dry weight than the control plants. Only the latter difference is statistically significant. Thus, in these treatments, the osmotic potential could be increased due to desiccation. The leaf water content of plants treated with either sodium chloride or calcium chloride have a water content greater than the control plants and hence greater than the other two treatments. The difference between the sodium chloride mean and the Carbowax mean at the 4 atm level is greater than the l.s.d. Thus, any observed increase in leaf osmotic potential in either the sodium or calcium chloride treatments probably is not the result of leaf desiccation.

Leaf area data are shown in Table XI. The total leaf area of the treated plants is decreased below the control plants in all but one case, that is, the intermediate level of treatment with sodium chloride. At the higher level of treatment all the treated plants show a reduced area as compared to the intermediate, 2 atm, level of treatment. The decrease in leaf area is more pronounced for plants treated with calcium chloride and sodium sulfate at the 4 atm level.

An indication of the distribution of leaf tissue and leaf thickness can be obtained by calculating the area per gram of dry weight of leaf tissue. Most of the treated plants at both levels have about the same value as the control plants. However, plants treated with sodium chloride and calcium chloride at the 2 atm level have greater area per gram of dry weight than the control. This also would indicate that the leaves on these plants would be thinner than the control leaves.

The average number of stomates per square centimeter (on the lower surface of the leaves) was measured and also is recorded in Table XI. Although all the treated plants have a slightly higher

TABLE XI

LEAF AREA AND STOMATAL DENSITY

Variable	Treatment	Level +2	Level +4
Total area of leaves per plant (One replicate) dm^2	Control	62.20	62.20
	NaCl	83.52	41.92
	$CaCl_2$	49.08	31.56
	Na_2SO_4	51.35	28.29
	CBWX	44.32	41.63
Total area per gram dry wt. of leaf tissue (One replicate) dm^2/gm	Control	3.95	3.95
	NaCl	8.75	3.56
	$CaCl_2$	4.30	3.29
	Na_2SO_4	3.51	3.33
	CBWX	2.98	3.33
Average stomatal density (Three replicates) Thousands of $stomates/cm^2$	Control	14.6 [1]	14.6
	NaCl	16.4	17.2
	$CaCl_2$	16.6	15.1
	Na_2SO_4	16.1	16.7
	CBWX	17.0	15.4

[1] The "t" test was applied to these data and none of the differences from the control mean was significant.

stomatal density than the control plants, the differences are not statistically significant.

3.122 Initiation and Expansion. The sum of the number of leaves on a plant is a measure of leaf initiation. By tagging the petioles at selected intervals it was determined when the leaves on each plant in the 8/22 harvest were first formed. A summary of these data is given in Table XII. It is obvious that inhibition of leaf initiation began as soon as the treatments were applied. All the treated plants have a total number of leaves less than the controls, and the number per plant decreases as the level of the treatment is increased. This is especially evident in the leaves initiated during the osmotic additions. Although the total number of leaves per plant decreased with treatment level, only the decrease for the sodium sulfate treatment is greater than the l.s.d. At the 4 atm level the Carbowax plants have a lower number of leaves than the control by a statistically significant amount. The Carbowax plants at this level also have more leaves than salt treated plants, but the difference is not statistically significant.

The average leaf size or the average area per leaf is a measure of the degree of leaf expansion, which is independent of leaf initiation. The data in Table XIII show that leaves already on the plant when treatment was initiated actually grew larger than the control, but leaves initiated after the nominal levels of the treatments had been attained were smaller in most cases. The exception is the calcium chloride

TABLE XII

NUMBER OF LEAVES PER PLANT
8/22 Harvest

Period of Initiation	Treatment	+2 atm Level				+4 atm Level				Analysis of Variance Results (Treatments Only)
					Mean				Mean	
7/18 - 8/9	Control	32	24	29	28	32	24	29	28	No significant variance.
	NaCl	22	27	38	29	30	23	29	27	
	$CaCl_2$	17	33	21	24	24	29	21	25	
	Na_2SO_4	32	22	--	27	22	19	21	21*	
	CBWX	28	30	26	28	33	20	30	28	
8/10 - 8/14	Control	59	51	56	55	59	51	56	55	Variance between columns significant at 5% level. F-Test
	NaCl	20	38	64	41	24	25	31	27**	
	$CaCl_2$	25	23	27	25**	28	28	24	27**	
	Na_2SO_4	34	46	--	40	30	16	21	22**	
	CBWX	41	42	31	38*	36	18	21	25**	
8/15 - 8/22	Control	89	68	41	66	89	68	41	66	No significant variance.
	NaCl	23	57	74	51	36	18	42	32	
	$CaCl_2$	22	49	30	34	15	26	29	23*	
	Na_2SO_4	38	50	--	44	15	22	23	20*	
	CBWX	23	63	50	45	25	50	60	45	
7/18 - 8/22	Control	180	143	126	150	180	143	126	150	Variance between columns significant at 5% level. F-Test
	NaCl	65	122	176	121	90	66	102	86*	
	$CaCl_2$	64	105	78	82*	67	83	74	75*	
	Na_2SO_4	104	118	--	111	67	57	65	63**	
	CBWX	92	135	107	111	94	88	111	98*	

* 5%)
** 1%) Level of significance Two Tail "t" test

TABLE XIII

AVERAGE AREA OF INDIVIDUAL LEAVES
(One Replicate) cm^2

8/22 Data

Period of Initiation	Treatment	Level +2	Level +4
7/18—8/9	Control	65.9	65.9
	NaCl	84.7	71.8
	$CaCl_2$	90.7	72.3
	Na_2SO_4	69.3	70.0
	CBWX	70.4	73.0
8/10—8/14	Control	63.3	63.3
	NaCl	60.3	52.3
	$CaCl_2$	83.3	51.8
	Na_2SO_4	65.9	55.0
	CBWX	57.8	53.3
8/15—8/22	Control	17.1	17.1
	NaCl	17.2	11.6
	$CaCl_2$	25.4	13.6
	Na_2SO_4	11.6	8.9
	CBWX	16.2	14.2
Total 7/18—8/22	Control	49.0	49.0
	NaCl	47.4	41.1
	$CaCl_2$	62.9	42.6
	Na_2SO_4	43.5	43.5
	CBWX	41.4	37.5

treated plants which developed larger leaves than the control plants at the 2 atm level. The average size of the leaves of the Carbowax treated plants at the 4 atm level is smaller than the control. In general, leaf expansion appears to be affected very little by the treatments.

A second experiment was conducted the following winter primarily to study changes in root characteristics. Several unusual characteristics appeared in the aerial portion of the plants. Some plants had petioles with only two leaflets, while others had four. In one case one leaflet and one flower developed on the same petiole. In another, all the leaflets on a petiole were merged into one leaf blade. These symptoms were most pronounced on the sodium sulfate treated plants but were also observed on plants treated with calcium chloride and sodium chloride. Plants in the control group or treated with Carbowax did not exhibit these malformations.

3.123 Net Assimilation Rates. The net assimilation rate, which is defined as the change in plant dry weight per unit area of leaf per day, is a measure of the net photosynthetic rate. The increase in total plant dry weight over a period of time is the best indication of the plant's utilization of the products of photosynthesis, and when this is expressed per unit area of leaf, it gives a measure of the photosynthetic efficiency of the leaves. The net assimilation rate was calculated for the period 8/6 through 8/22, and the results are shown in Table XIV. The net assimilation rate for the control plants is greater than for any

TABLE XIV

NET ASSIMILATION RATE (One Replicate)
Dry Weight Production/Day/Unit Leaf Area

$gm/da/m^2$

Treatment	+2 atm Level			+4 atm Level			Analysis of Variance Results (Treatments Only)
	8/6 - 8/14	8/14 - 8/22	Mean	8/6 - 8/14	8/14 - 8/22	Mean	
Control	7.14	6.34	6.74	7.14	6.34	6.74	
NaCl	5.88	2.86	4.37	4.71	3.13	3.92	No Significant Variance
$CaCl_2$	5.26	4.42	4.84	5.23	2.67	3.95	
Na_2SO_4	4.76	5.91	5.34	4.75	3.26	4.00	
CBWX	4.86	7.73	6.30	7.16	5.58	6.37	

Note: "t" test showed no significant differences between control means and individual treatment means.

of the treated plants. Carbowax treated plants had net assimilation rates less than the control, but more than any of the salt treated plants, which tended to respond about equally. The net assimilation rate decreased as the level of the treatments increased. None of these differences are statistically significant.

3.13 Root Growth and Metabolism

3.131 Physical Characteristics. The physical characteristics of the roots harvested on 8/22 are shown in Table XV. The dry weight of the roots of all the treated plants is below the value for the control plants. The dry weight decrease is less in the Carbowax treated plants than in the salt treated plants. At both levels the Carbowax plants' dry weight is greater than each of the salt treatments by an amount exceeding the l.s.d. The root growth inhibition of plants treated with Carbowax and sodium sulfate increases with the level of the treatments; but in plants treated with sodium chloride or calcium chloride, the inhibition caused by the 2 atm treatment is as severe as that caused by the 4 atm treatment. The differences between the means at 2 atm and 4 atm for sodium sulfate and Carbowax is greater than the l.s.d. The order of treatments causing increasing growth inhibition at the 4 atm level is control, Carbowax, calcium chloride, sodium chloride and sodium sulfate.

The decrease in the water content of the roots is the most statistically significant measure of growth inhibition in the roots. As with

TABLF XV

PHYSICAL CHARACTFRISTICS OF ROOTS
8/22 Harvest

Variable	Treatment	+2 atm Level				+4 atm Level				Analysis of Variance Results (Treatments Only)
					Mean				Mean	
	Control	4.57	7.18	4.06	5.27	4.57	7.18	4.06	5.27	Variance between
Dry Weight/	NaCl	3.20	2.60	---	2.90	2.60	2.60	2.50	2.57*	rows significant at
Plant	$CaCl_2$	2.70	2.02	4.20	2.95	3.16	2.78	3.30	3.08*	1% level. Variance
gm	Na_2SO_4	3.20	3.56	---	3.38	2.28	1.75	2.19	2.07*	between columns,
	CBWX	4.59	4.61	4.75	4.65	4.26	2.43	3.60	3.43	significant at 5%
										level. F-Test
	Control	72.54	92.87	73.25	79.55	72.54	92.87	73.25	79.55	Variance between
Water Con-	NaCl	57.80	54.80	---	56.30*	50.37	54.80	34.00	46.39**	columns signifi-
tent/Plant	$CaCl_2$	34.40	39.98	50.58	41.65**	39.03	29.98	33.35	34.12**	cant at 1% level.
ml	Na_2SO_4	59.04	71.21	---	65.12	34.84	24.75	29.93	29.84**	F-Test
	CBWX	53.21	58.00	56.20	55.80**	53.85	23.06	40.00	38.97**	
	Control	15.88	12.93	18.04	15.62	15.88	12.93	18.04	15.62	Variance between
Water/Dry	NaCl	18.06	21.08	---	19.57	19.37	21.08	13.60	18.02	rows significant at
Weight	$CaCl_2$	12.74	19.79	12.04	14.86	12.35	10.78	10.11	11.08*	1% level. Variance
ml/gm	Na_2SO_4	18.45	20.00	---	19.23	15.28	14.14	13.67	14.36	between columns
	CBWX	11.59	12.58	11.83	12.00*	12.64	9.49	11.11	11.08	significant at 5%
										level.

* 5%)
** 1%) Significance of One-Tail "t" test

dry weight, the increase in treatment level in the sodium sulfate and Carbowax treatments brings about a decrease in water content greater than the l. s. d. But in general the decrease in water content is similar, but not in the same proportion, to the decrease in dry weight. Consequently, the ratios of water content to dry weight are not constant. These data, also in Table XV, show that roots growing in solutions with sodium chloride or sodium sulfate added, generally have a higher water content per unit of dry weight than the roots in any other treatment, including the control. The difference of both these treatments from the Carbowax treatment is greater than the l. s. d. at the 2 atm level, but at the 4 atm level this is true of only sodium chloride. These results are caused by a reduction in dry matter content which is greater than the reduction in the water content of the roots in these treatments. Conversely, the water content is reduced more than the dry matter content of roots in solutions of calcium chloride or Carbowax. The water content per dry weight of the sodium sulfate treated roots is not significantly different than the Carbowax treatment at the 4 atm level, since the greatest decrease between treatment levels occurred in the sodium sulfate treatment. The difference between the 2 atm mean and the 4 atm mean in the sodium sulfate treatment is greater than the l. s. d.

3.132 Respiration Rates. The respiration rates of root tips were measured on 8/14 and 8/22. The former observation period was two days after the 4 atm treatments reached their final level, and the latter was after the roots had been growing in solutions at the nominal levels of treatment for more than one week. The results of the measurements are shown in Table XVI. The respiration rates of roots growing in solutions with sodium chloride or sodium sulfate added is increased by increasing the level of the treatment. The magnitude of this increase was great enough on 8/22 that in these treatments the respiration rate exceeded the respiration rate of the roots in the control solution. On 8/22 the Carbowax treated roots had a respiration rate only slightly below the control rate. While this also was true for calcium chloride at the 2 atm level, it was not true for this salt at the 4 atm level where the respiration rate was about one-half that of the control roots.

3.133 Morphological Changes. Changes in root morphology were observed on two scales. On the gross scale, roots of the treated plants had more adventitious roots emerging from the transition zone between the roots and the stem. The primary root system of the treated plants was stunted, thus leaving an open area with no roots directly below the stem. On a finer scale, the root tips also showed morphological differences. A full scale drawing of typical root tip samples from each treatment and the control plants is shown in Figure 6. Notice that the initiation of secondary roots is nearer the root tip in the treated plants, especially in the salt treatments. The gradual

TABLE XVI

ROOT RESPIRATION RATE (One Replicate)
Oxygen consumed/unit time/unit dry weight

μl /min/gm

Date	Treatment	+2 atm	+4 atm
	Control	232.0	232.0
	NaCl	129.6	168.6
8/14	$CaCl_2$	48.0	109.4
	Na_2SO_4	202.0	349.3
	CBWX	178.1	194.3
	Control	80.8	80.8
	NaCl	138.9	192.6
8/22	$CaCl_2$	73.8	40.0
	Na_2SO_4	122.0	215.6
	CBWX	65.9	70.8
	Control	156.4	156.4
	NaCl	134.2	180.6
Mean	$CaCl_2$	60.9	74.7
	Na_2SO_4	162.0	282.4
	CBWX	122.0	132.6

"t" test shows that means of individual treatments are not significantly different from the control mean.

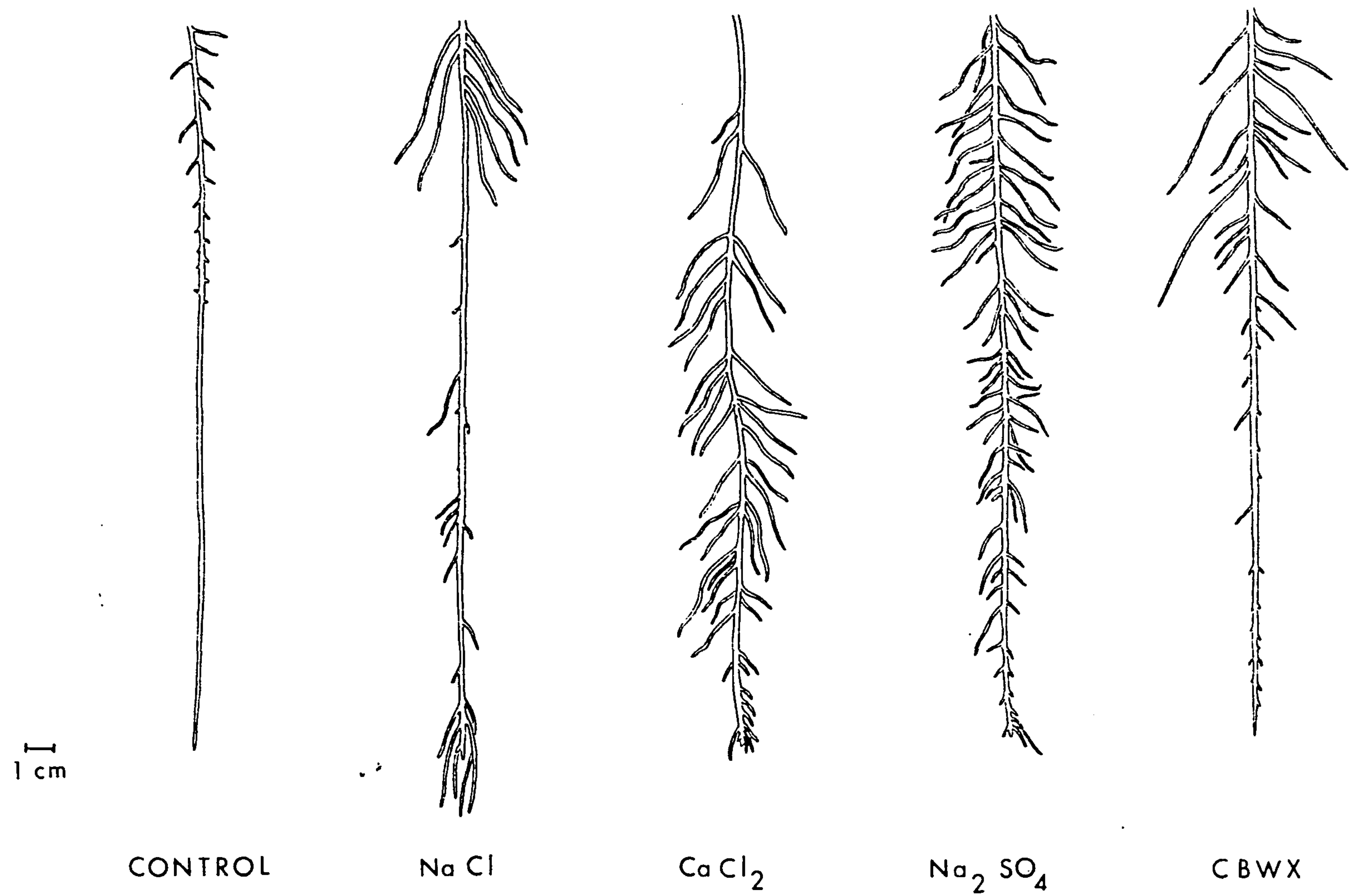
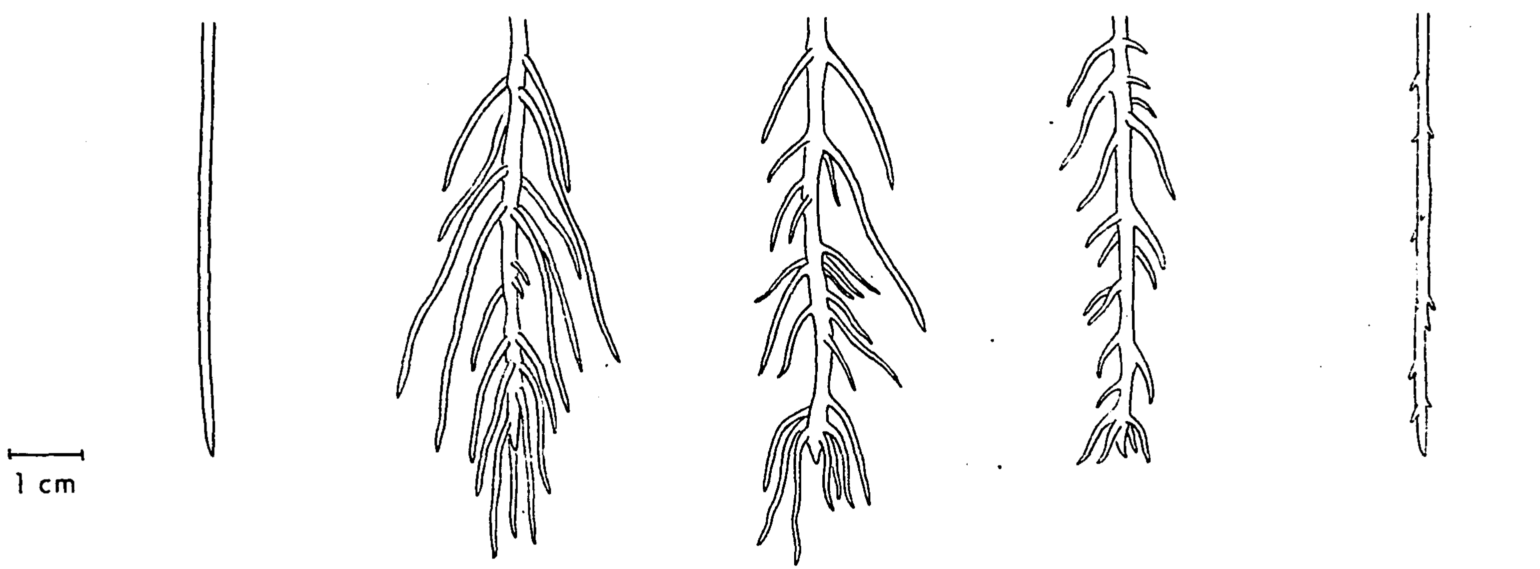


Fig. 6.--Morphological Characteristics of Root End Segments Scale XI (Drawing by Sara Fish)

development of longer and longer secondary roots progressed regularly up the primary root in the control plants even though the longer secondary roots are not shown in the illustration. In Figure 7 typical root tips from each of the treatments and the control are shown at a magnification twice normal size. The tip of the control plant root shows no signs of branching in this region. A few secondary roots have been initiated on the roots of Carbowax treated plants. The salt treated plants have extensive secondary roots. In the sodium chloride and the calcium chloride treatments, the secondary roots show more elongation than in the sodium sulfate treatments.

3.2 Water Metabolism

3.21 Transpiration. The average daily transpiration per plant is shown in Table XVII for three growth periods during the experimental period. The periods represent the following conditions: 8/6-8/12, osmotic levels of solutions increased to nominal levels; 8/13-8/16, nominal osmotic levels maintained prior to nutrient change; 8/17-8/22, osmotic levels maintained after nutrient change. During each period the treated plants transpired less than the control plants. At the 2 atm level of treatment, transpiration from calcium chloride and sodium chloride treated plants was the lowest of all the treatments. The difference between these treatments and the Carbowax treatments was greater than the l.s.d. in the 8/17-8/22 period of growth and in the total period of



CONTROL | NaCl | $CaCl_2$ | Na_2SO_4 | CBWX

Fig. 7.--Morphological Characteristics of Root Tips Scale X2 (Drawing by Sara Fish)

TABLE XVII

AVERAGE DAILY TRANSPIRATION RATE PER PLANT

ml/da/Plant

Time Interval	Treatment	Level +2	Level +4	Analysis of Variance Results (Treatment only)
	Control	251.6		
	NaCl	195.7*	207.9	No significant
8/6—8/12	$CaCl_2$	193.7*	203.7	differences in
	Na_2SO_4	210.6	192.9*	variance
	CBWX	223.5	228.1	
	Control	517.4		
	NaCl	296.0*	252.2**	Variance between
8/13—8/16	$CaCl_2$	227.3**	179.1**	columns signifi-
	Na_2SO_4	320.2**	243.1**	cant at 1% level
	CBWX	318.5*	223.6**	F-Test
	Control	501.2		
	NaCl	208.9**	154.9**	Variance between
8/17—8/22	$CaCl_2$	194.5**	89.3**	columns signifi-
	Na_2SO_4	271.2*	183.0**	cant at 1% level
	CBWX	323.6	110.8**	F-Test
	Control	402.2		Variance between
	NaCl	224.0**	204.7**	rows significant
8/6—8/22	$CaCl_2$	202.0**	157.5**	at 5% level. Vari-
	Na_2SO_4	257.8**	211.7**	ance between
	CBWX	281.2*	185.6**	columns signifi-
				cant at 1% level
				F-Test

* 5%)
** 1%) Significance One Tail "t" test

Each value in the table is the average of the daily transpiration for the indicated period. Daily transpiration values are the average values of at least four observations.

growth. At the 4 atm level of treatment, calcium chloride and Carbowax had the lowest daily transpiration, except in the initial period during the osmotic additions. The first period was also the exception to the general trend found in the other periods that the transpiration decreased with increasing treatment levels. The most significant decrease with treatment level was in the Carbowax treatment which had a difference between the 2 atm transpiration and the 4 atm transpiration greater than the l. s. d., except in the first period.

By combining the leaf area measurements with the daily transpiration for the same plants, the average daily transpiration per unit area of leaf was calculated. These data are shown in Table XVIII. In most cases the transpiration per leaf area for the treated plants is below the values for the control plants. It is interesting to note that for the earlier period, comprising a period slightly longer than the period of osmotic additions, the transpiration per unit leaf area is lowest for the Carbowax treatment and next lowest for the sodium sulfate treatment. During the period when osmotic levels were maintained at their nominal levels these treatments have the highest transpiration per unit area. In addition there is a trend in the latter period, but not so obvious in the former period, for the transpiration per unit leaf area to increase with increasing levels of treatment for the salt treated plants and to decrease with increasing levels of treatment for the Carbowax treatments. With only

TABLE XVIII

MEAN DAILY TRANSPIRATION PER LEAF AREA
(One Replicate)

$ml/dm^2/da$

Time Interval	Treatment	Level +2	Level +4
	Control	15.30	15.30
	NaCl	13.90	12.65
8/6—8/14	$CaCl_2$	13.50	12.69
	Na_2SO_4	11.85	12.56
	CBWX	10.93	7.29
	Control	7.71	7.71
	NaCl	4.14	5.68
8/15—8/22	$CaCl_2$	4.85	5.58
	Na_2SO_4	6.60	8.30
	CBWX	8.77	7.23

one replicate, the statistical significance of these differences can not be determined.

3.22 Resistance of Plant to Water Movement

3.221 Root Resistance. The resistance to water movement through the roots of de-topped plants was measured on 8/13 and on 8/21. The plants selected on 8/13 were at 3 atm of treatment and those analyzed on 8/21 were at 4 atm of treatment. The data are shown in Table XIX. High values of root resistance indicate a high resistance to water movement or a low tissue permeability. On 8/13 all the treated plants had a higher root resistance than the controls. Sodium chloride and calcium chloride have slightly greater values than the other treatments. On 8/21 the sodium sulfate treated plants have the highest root resistance with a value nearly 10 times the root resistance of the control plants. The resistance of roots in the Carbowax treatment is much less than that in sodium sulfate but slightly greater than the resistance of sodium chloride and calcium chloride treated plants.

The magnitude of the treatment effects on root resistance was much greater than expected; thus a second experiment was conducted during the following winter to see if additional observations would give similar results. The design, procedures, and treatment levels of the winter experiment were the same as described in Chapter 2. The time variation of resistance during the winter experiment is shown in Figure 8. The relative order of the resistance increase according to treatments

TABLE XIX

ROOT RESISTANCE TO WATER MOVEMENT
Root Fresh Weight/Flow Rate at 15 PSI
(One Replicate)
gm/ml/hr

Treatment	+3 atm 8/13	+4 atm 8/21
Control	3.11	4.77
NaCl	8.46	13.58
$CaCl_2$	8.77	9.61
Na_2SO_4	7.33	40.53
CBWX	7.37	18.60

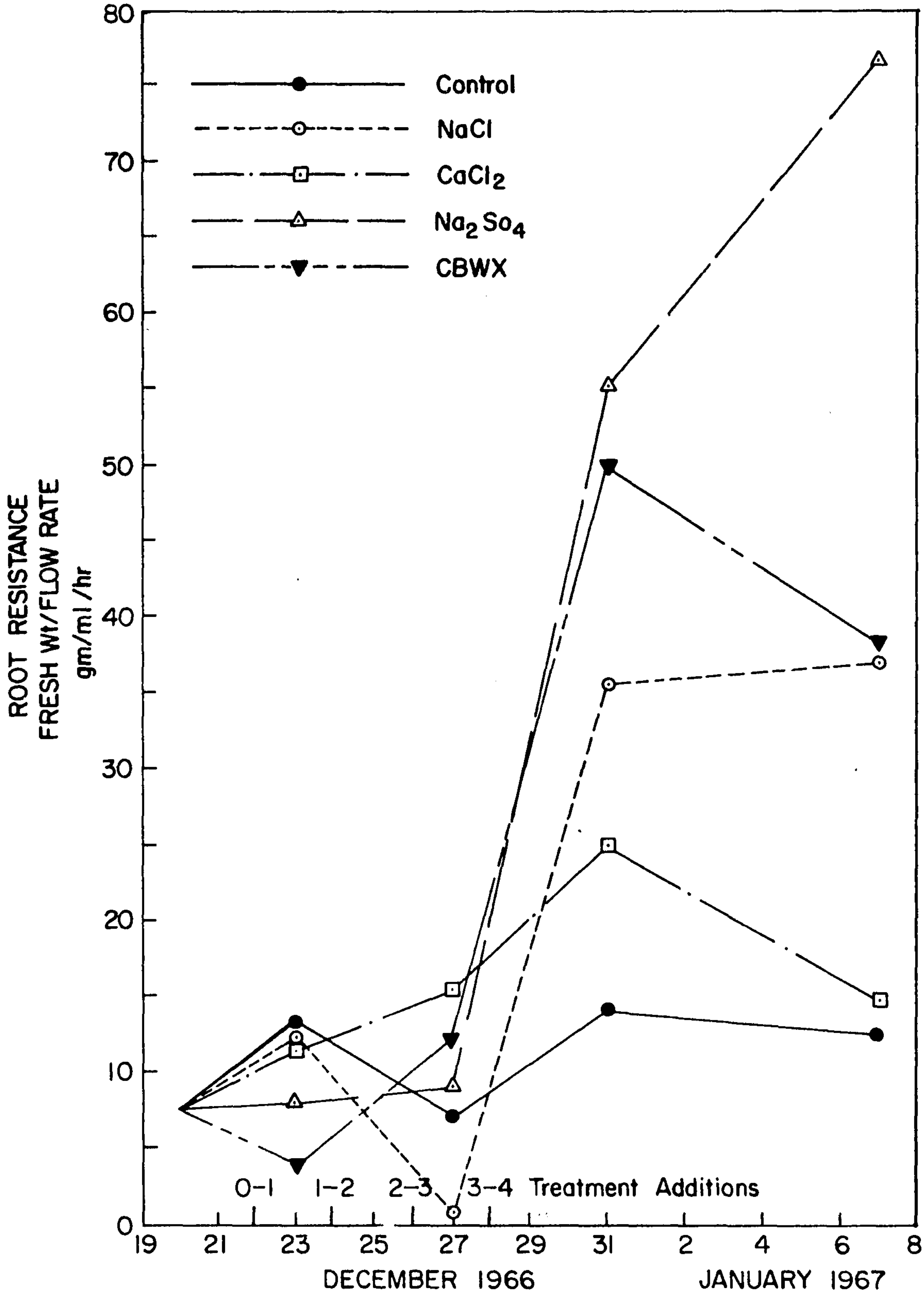


Fig. 8.--Root Resistance to Water Movement as a Function of Time (Winter 1966 Data) 4 atm Treatments

after the plants have reached the 4 atm level is the same as in the summer data. The reason for the decrease in root resistance values in the calcium chloride and Carbowax treatments near the end of the experimental period is due to the fact that the plants in these treatments were severely damaged by the treatments and by the last observation period they were near death. The plants had brittle stems and it was difficult to get an adequate seal around the stem without cracking it and letting water flow in the hollow stem.

In Figure 9 and Table XX the winter data taken after the 4 atm treatments reached their nominal level were averaged to obtain the variation of root resistance with level of treatment. It is apparent that all the treatments showed some increase in resistance over the control. At the 2 atm level the calcium chloride treated plants had a resistance greater than Carbowax, by an amount exceeding the l. s. d. The decreases in resistance of the calcium chloride roots with the level of treatment, which is greater than the l. s. d., is due to the severe damage caused to the plant at the 4 atm level. The sodium sulfate treated plants showed the largest increase in resistance with level of treatment. The difference between the 2 atm and 4 atm sodium sulfate treatments is greater than the l. s. d. At the 4 atm level the resistance of the sodium sulfate treated roots is greater than the Carbowax plants' resistance by an amount greater than the l. s. d., while the

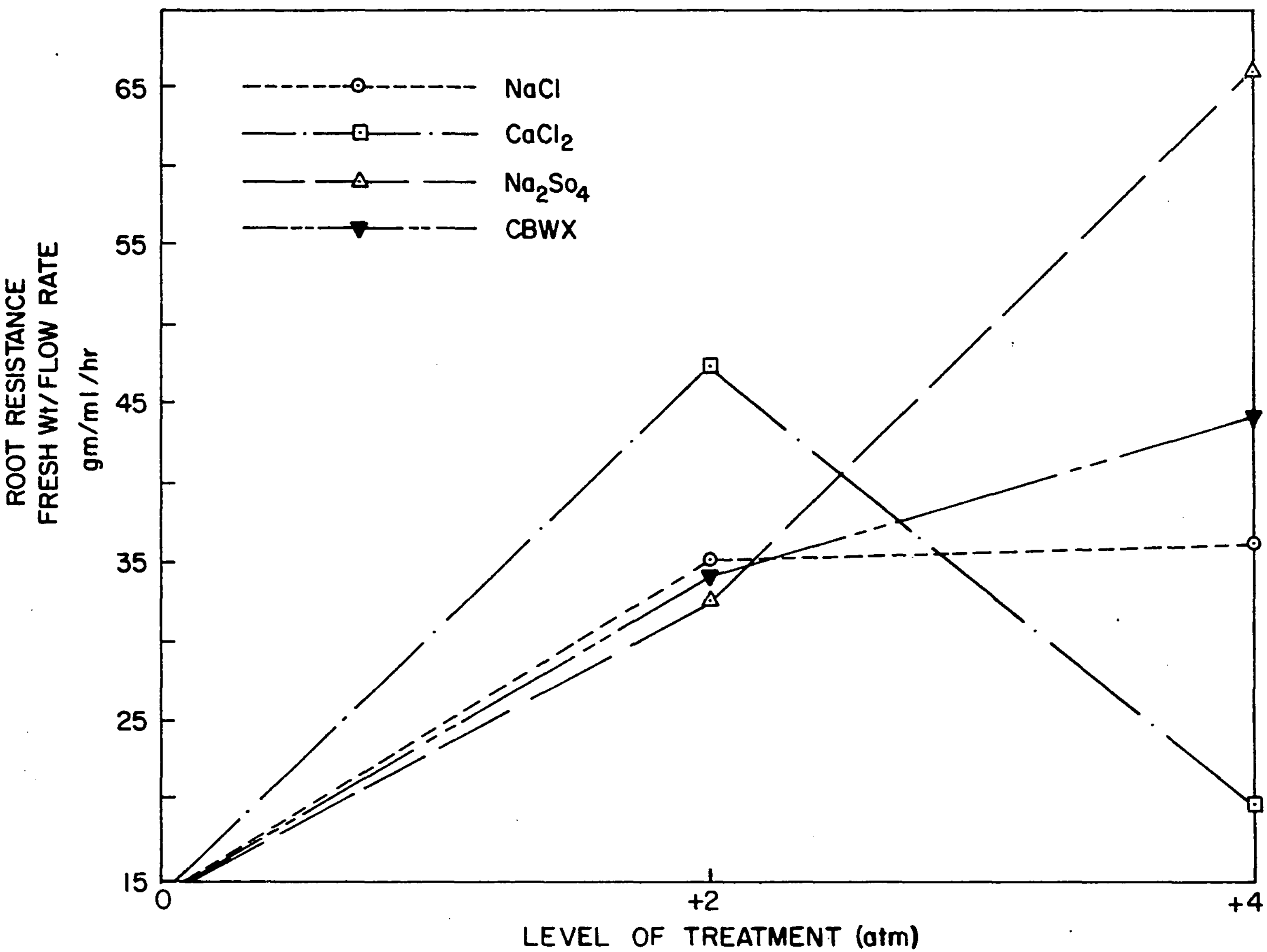


Fig. 9.--Root Resistance to Water Movement as a Function of Treatment Level (Average of Winter Data Taken After 12/27/66)

TABLE XX

AVERAGE VALUE OF ROOT RESISTANCE TO WATER MOVEMENT (WINTER DATA)
Fresh Wt/Flow Rate at 15 PSI

gm/ml/hr

Treatment	Level		Results of Analysis of Variance (Treatments Only)
	+2	+4	
Control	15.0	13.4	
NaCl	35.2	36.4**	
$CaCl_2$	47.8**	20.0	Only Interaction Significant
Na_2SO_4	32.8**	66.2*	
CBWX	34.3*	44.2*	

* 5%)
** 1%) Level of significance One Tail "t" test

Note: Data in Table are averages of two readings taken after plants had grown in full level treatments for extended period.

calcium chloride treated roots' resistance was below the Carbowax plants' resistance by an amount exceeding the l.s.d.

3.222 Leaf Diffusion Resistance. The leaf diffusion resistance is a measure of the resistance a leaf offers to the diffusion of water vapor from the mesophyll to the atmosphere. A positive value of resistance indicates that the rate of diffusion of water vapor is greater than that for a saturated evaporating surface under the same ambient conditions. The leaf diffusion resistance data shown in Table XXI, indicate that all the leaves of the treated plants, after reaching their final treatment level, have a resistance to water vapor diffusion greater than the control leaves. The leaves of the Carbowax treated plants have the highest leaf diffusion resistance. The mean of the Carbowax treatment is greater than each of the salt treatments by an amount exceeding the l.s.d. In addition, the mean of the sodium sulfate treatment exceeds the mean of both the sodium chloride and the calcium chloride treatments by an amount exceeding the l.s.d.

3.23 Water Energy Observations

3.231 Osmotic Potential. The osmotic potential of the expressed leaf sap was estimated from conductivity measurements. The relationship between the osmotic potential and conductivity for the pure salts of sodium chloride, calcium chloride, and sodium sulfate was used for the corresponding treatments. The relationship for the control nutrient solution was used for the Carbowax treatments since

TABLE XXI

LEAF RESISTANCE TO WATER VAPOR DIFFUSION
4 Atm. Treatments

sec/cm

Date	Treatment	+4 Atm. Level				Analysis of Variance Results (Treatments Only)
					Mean	
	Control	11.13	8.72	12.61	10.82	No statistical tests
	NaCl	0.76	12.01	17.09	9.95	run since plants had
8/7	$CaCl_2$	18.15	11.37	3.14	10.89	not reached final
	Na_2SO_4	6.19	1.26	3.56	3.67	treatment level.
	CBWX	1.86	8.25	9.57	5.89	
	Control	1.12	2.26	0.62	1.33	No statistical tests
	NaCl	3.86	2.86	2.90	3.21	run since plants had
8/11	$CaCl_2$	27.88	15.26	9.17	17.44	not reached final
	Na_2SO_4	25.74	3.08	4.35	12.66	treatment level.
	CBWX	4.26	2.34	4.94	3.85	
	Control	6.61	4.99	3.30	4.97	8/15, 8/18, 8/20
	NaCl	29.21	27.38	39.22	31.94	Data combined for
8/15	$CaCl_2$	24.35	27.33	21.98	24.55	analysis of variance.
	Na_2SO_4	28.87	37.24	61.66	42.59	Variance between rows,
	CBWX	96.35	159.86	97.42	117.88	columns and interaction
						significant at 1% level.

(Table Continued on Next Page)

TABLE XXI--Continued.

Date	Treatment	+4 Atm. Level				Analysis of Variance Results (Treatments Only
					Mean	
	Control	10.21	5.68	1.44	5.78	
	NaCl	20.36	12.12	15.67	16.05	
8/18	$CaCl_2$	14.57	10.85	11.92	12.45	8/15, 8/18, 8/20
	Na_2SO_4	58.79	38.25	61.95	53.00	Data combined for
	CBWX	28.10	5.45	40.48	24.68	analysis of variance.
						Variance between rows,
	Control	2.59	5.98	2.63	3.73	columns and interaction
	NaCl	40.52	29.57	13.22	27.77	significant at 1% level.
8/20	$CaCl_2$	39.52	38.01	11.18	29.57	
	Na_2SO_4	59.20	33.15	22.45	38.27	
	CBWX	21.64	9.60	18.50	16.58	
	Control				4.82	
Mean	NaCl				25.25**	
8/15—8/20	$CaCl_2$				22.19**	
	Na_2SO_4				44.62**	
	CBWX				53.04**	

** "t" test shows significance at 1% level.

conductivity is not altered by Carbowax. Since Carbowax can be taken into the plant, the osmotic potential calculated from the sap conductivity data for this treatment may be underestimated.[1] The calculated leaf sap osmotic potentials are shown in Table XXII.

All the leaves from the treated plants have higher osmotic potentials than leaves from the control plants. The sodium chloride treatments show the greatest increase in osmotic potentials with time and Carbowax the least.

The osmotic potential gradient can be calculated by taking the difference between the osmotic potential of the leaf sap and the osmotic potential of the growth solution. The changes in the osmotic potential gradient with time are shown in Figure 10. During the period of osmotic additions to the growth solution, all the treatments except the control and sodium chloride show a rapid decrease in the osmotic potential gradient. Sodium chloride treated plants show a slight decrease during this initial period but are able to recover and attain a gradient nearly equal to the gradient for the control plants. The other treatments show little osmotic adjustment and thus are never able to regain their original osmotic potential gradient.

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1. No quantitative data were taken on Carbowax uptake, but the decrease in the osmotic potential of the growth solution was greater than could be accounted for by removal of ions.

TABLE XXII

OSMOTIC POTENTIAL OF LEAF SAP
4 atm Treatments (One Replicate)

atm

Date	Control	NaCl	$CaCl_2$	Na_2SO_4	CBWX
8/7	4.0	4.6	3.8	4.3	4.3
8/9	4.6	6.5	4.5	4.8	4.7
8/11	4.2	6.2	4.5	4.7	5.0
8/13	3.9	6.2	5.0	5.6	5.0
8/21	4.5	8.1	6.5	6.6	5.4
Mean	4.24	6.32**	4.86	5.20*	4.88**

* Significant at 5% level)
** Significant at 1% Level) One Tail "t" test paired data

Note: Leaf osmotic potential calculated from leaf sap conductivity data.

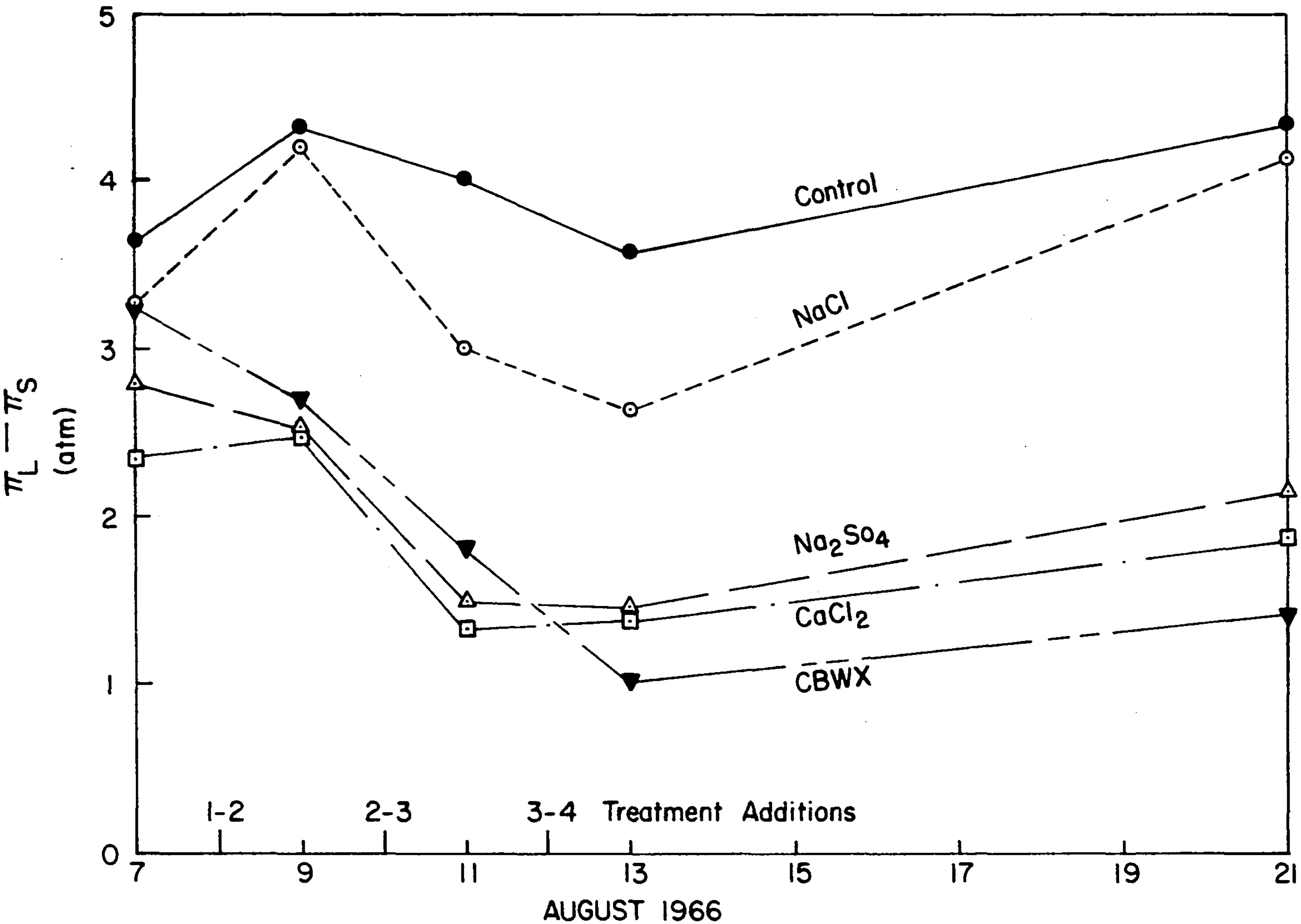

Fig. 10.--Difference Between the Osmotic Potential of the Leaf Sap (Π_L) and the Growth Solution (Π_S) as a Function of Time. 4 atm Treatments

Figure 11 shows the osmotic potential gradient variation with the level of treatment on 8/21. Sodium chloride shows little decrease with increasing level of treatment, but the other treatments show a nearly linear decrease of osmotic potential gradient as the level of treatment is increased.

3.232 Water Potential. The water potential of the leaves of each of the treatments is shown in Table XXIII. Sodium sulfate and Carbowax treated plants show an increase in the absolute value of the water potential while the other treatments show little change. Figure 12 shows the variation with time of the water potential gradient from the growth solution to the leaves. Plants with the greatest difference in water potential have the highest driving force for moving water through the plant. All the treated plants show a decrease in water potential gradient with the greatest decrease in the sodium chloride and calcium chloride treatments and the least in the Carbowax treatments.

The variation of the water potential gradient, from the growth solution to the leaves, with level of treatment on 8/21, is shown in Figure 13. All the treatments show a nearly linear decrease in water potential gradient with increasing level of treatment. Calcium chloride and sodium chloride show the greatest decrease and Carbowax the least.

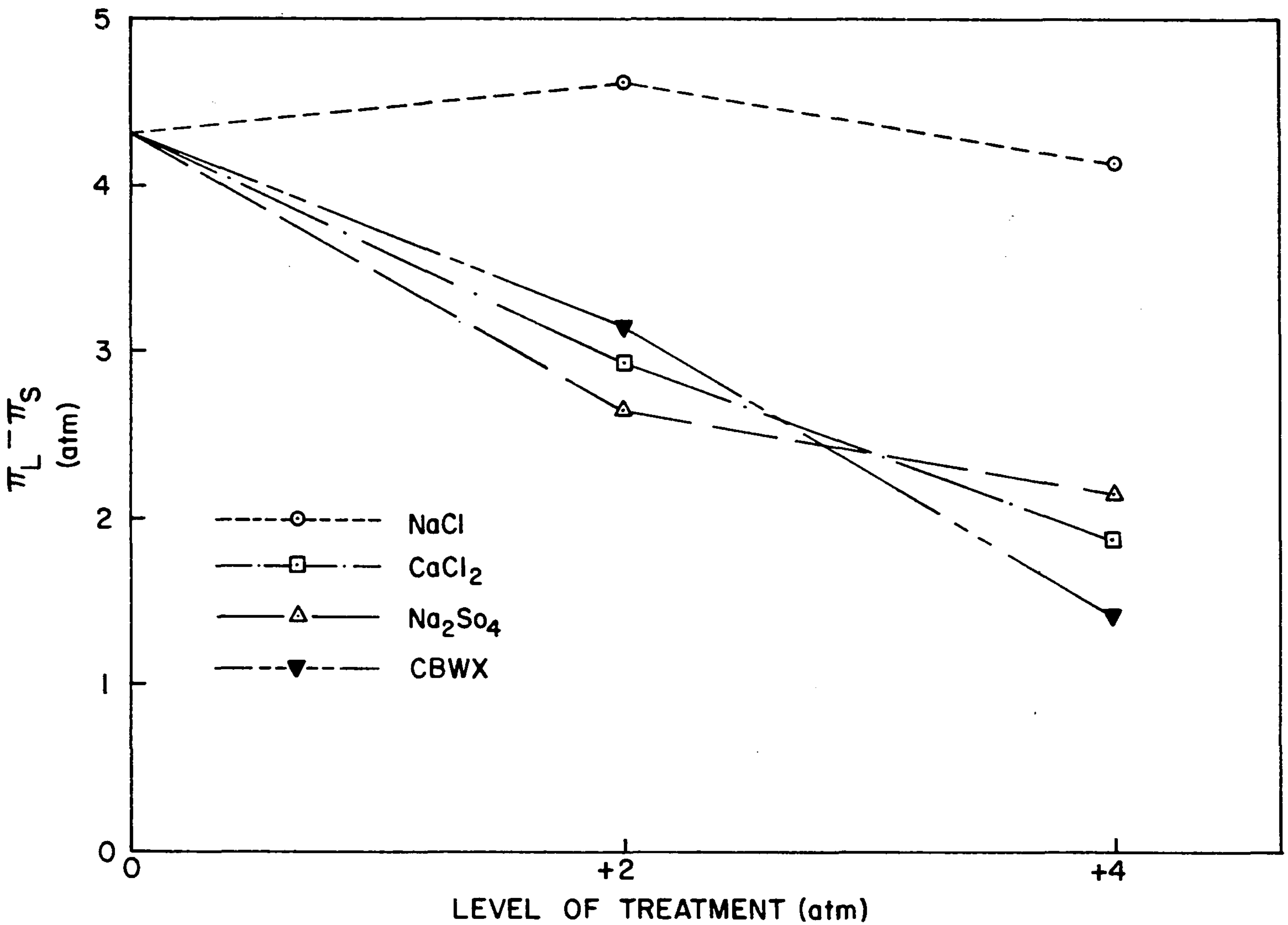

Fig. 11.--Difference Between the Osmotic Potential of the Leaf Sap (Π_L) and the Growth Solution (Π_S) as a Function of the Level of Treatment (8/21/66 data)

TABLE XXIII

LEAF WATER POTENTIAL OBSERVATIONS
4 atm Treatments (One Replicate)

atm

Date	Control	NaCl	$CaCl_2$	Na_2SO_4	CBWX
8/7	-4.0	-4.5	-7.0	-6.5	-5.5
8/9	-6.5	-5.5	-4.5	-7.3	-7.0
8/11	-6.5	-7.0	-7.0	-7.5	-7.5
8/13	-7.0	-7.0	-6.5	-8.5	-9.0
8/21	-7.5	-7.0	-7.0	-9.5	-10.5
Mean	-6.30	-6.20	-6.40	-7.86**	-7.90*

* Significant at 5% level) One Tail "t" test for paired data
** Significant at 1% level)

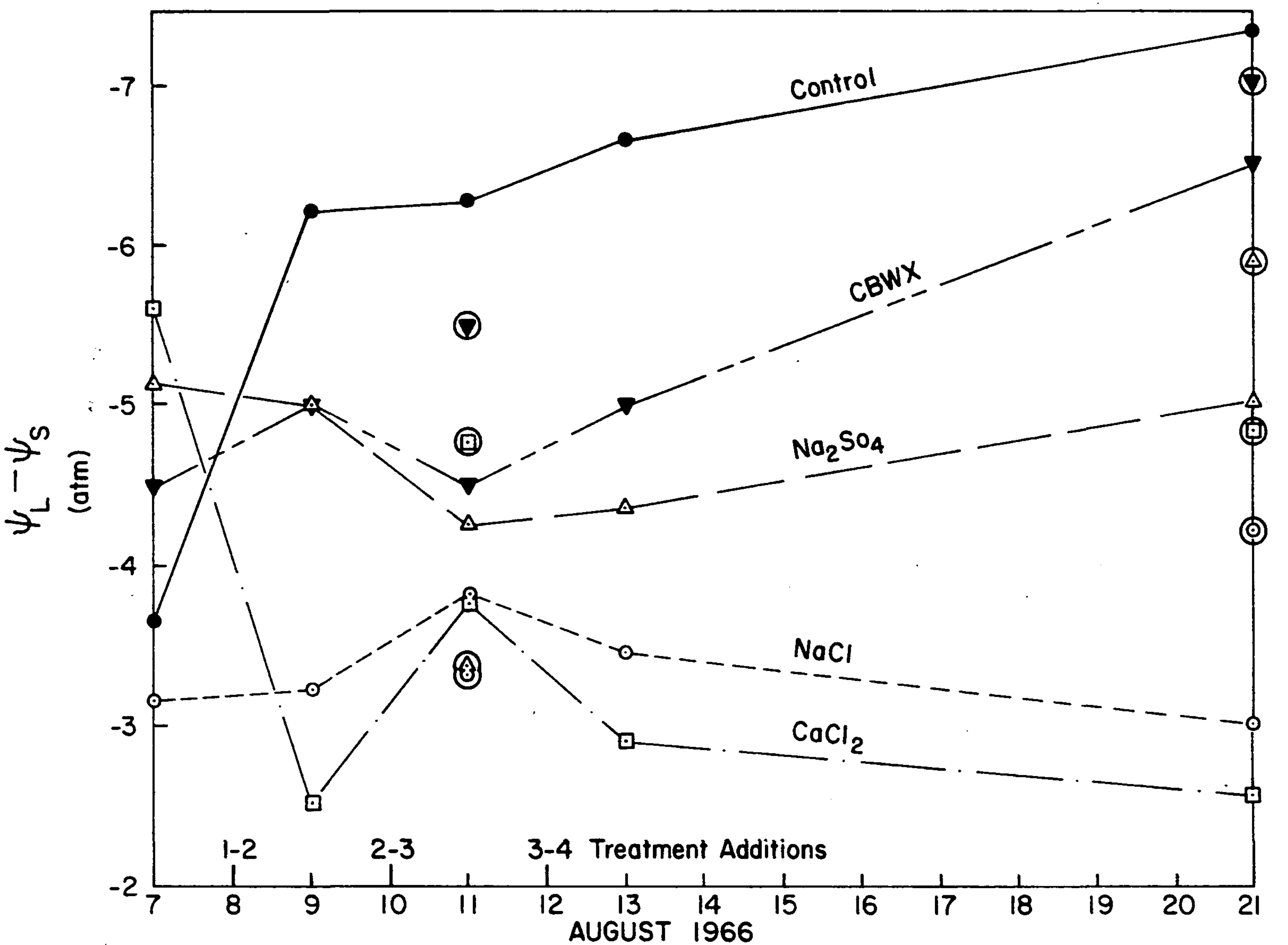

Fig. 12.--Time Variation of the Water Potential Gradient Between the Leaves (ψ_L) and the Growth Solution (ψ_S). 4 atm treatments (2 atm data shown with large circles around data points)

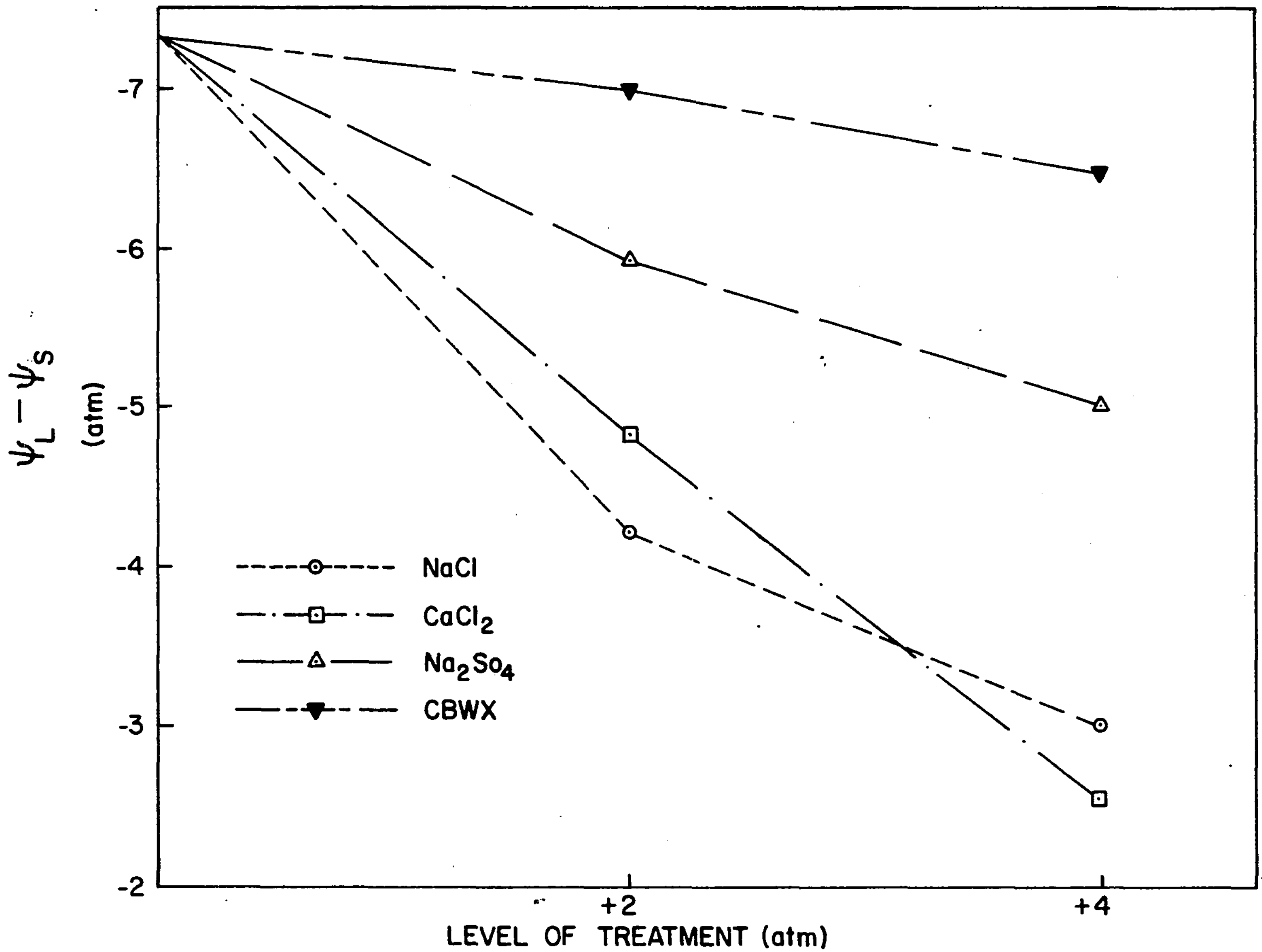

Fig. 13.--Gradient of Water Potential Between the Leaves (Ψ_L) and the Growth Solution (Ψ_s) as a Function of the Level of Treatment (8/21/66 data)

3.3 Ion Uptake Observations

3.31 Cumulative Uptake. The cumulative daily uptake of the cations, sodium, potassium, and calcium and magnesium for each of the treatments at the 4 atm level and the control treatment are shown in Figures 14 through 18. These data were obtained by calculating the rate of disappearance of the ions from the growth solutions. Each point is calculated from the average of usually four concentration observations. In treatments where either sodium or calcium was added, the uptake scale is X10 for the added ion and is shown on the right ordinate.

The cumulative uptake of potassium and magnesium, and sodium and calcium when they were not added as part of a treatment, was greatest for the control plants. In general, calcium uptake exceeded potassium and potassium exceeded magnesium which in turn usually exceeded sodium (except in the sodium chloride and sodium sulfate treatments).

Plants treated with sodium chloride took up little sodium until after the 3 atm level was reached, and then uptake increased rapidly. After 8/18 there was little uptake of calcium or potassium and there was a loss of magnesium from the plants.

Plants treated with calcium chloride began to take up calcium at a high rate as soon as the treatment was initiated. The high calcium accumulation, which continued until the plant growth solutions were brought to 4 atm, tended to suppress the potassium and magnesium

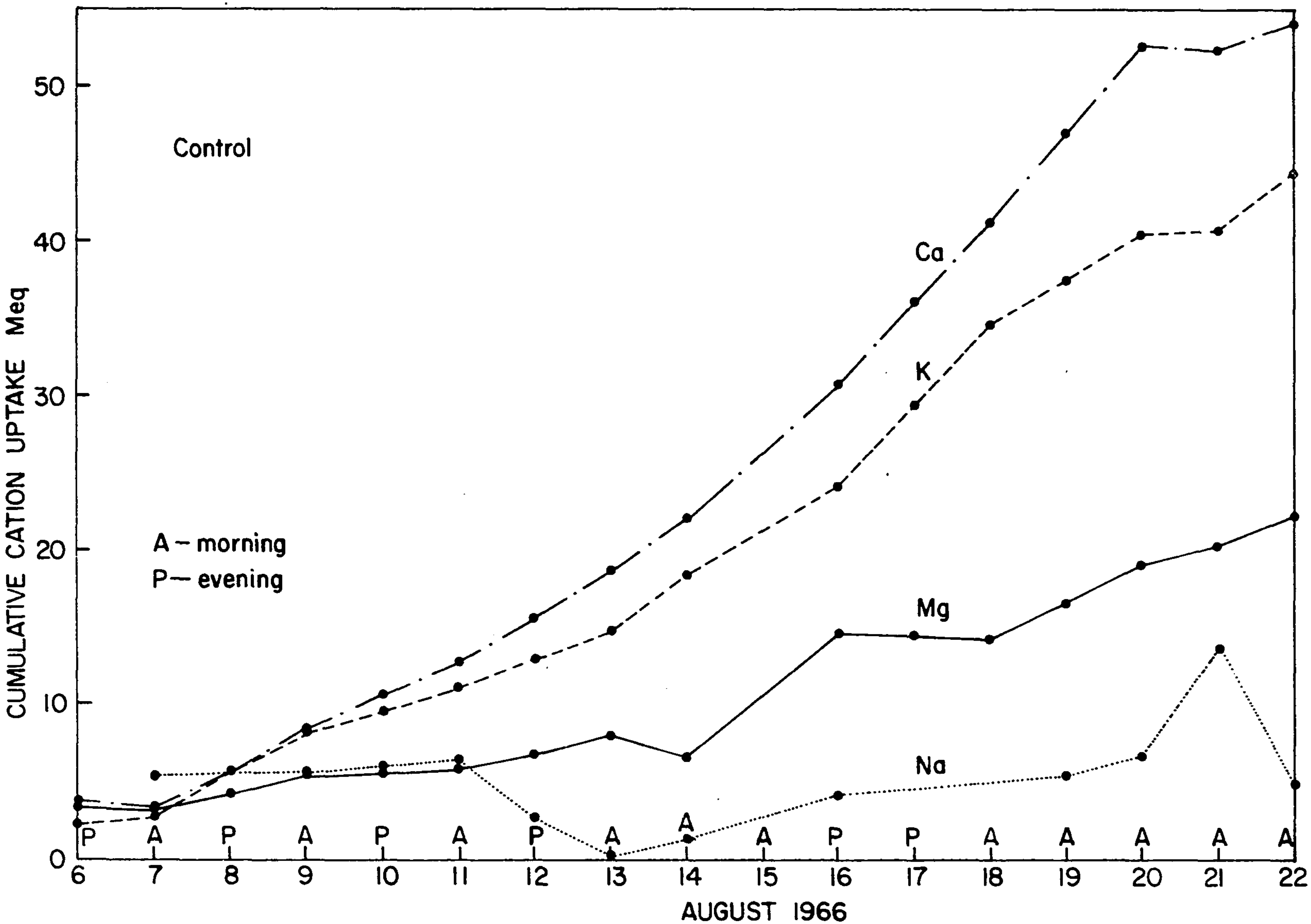

Fig. 14.--Cumulative Cation Uptake-Control

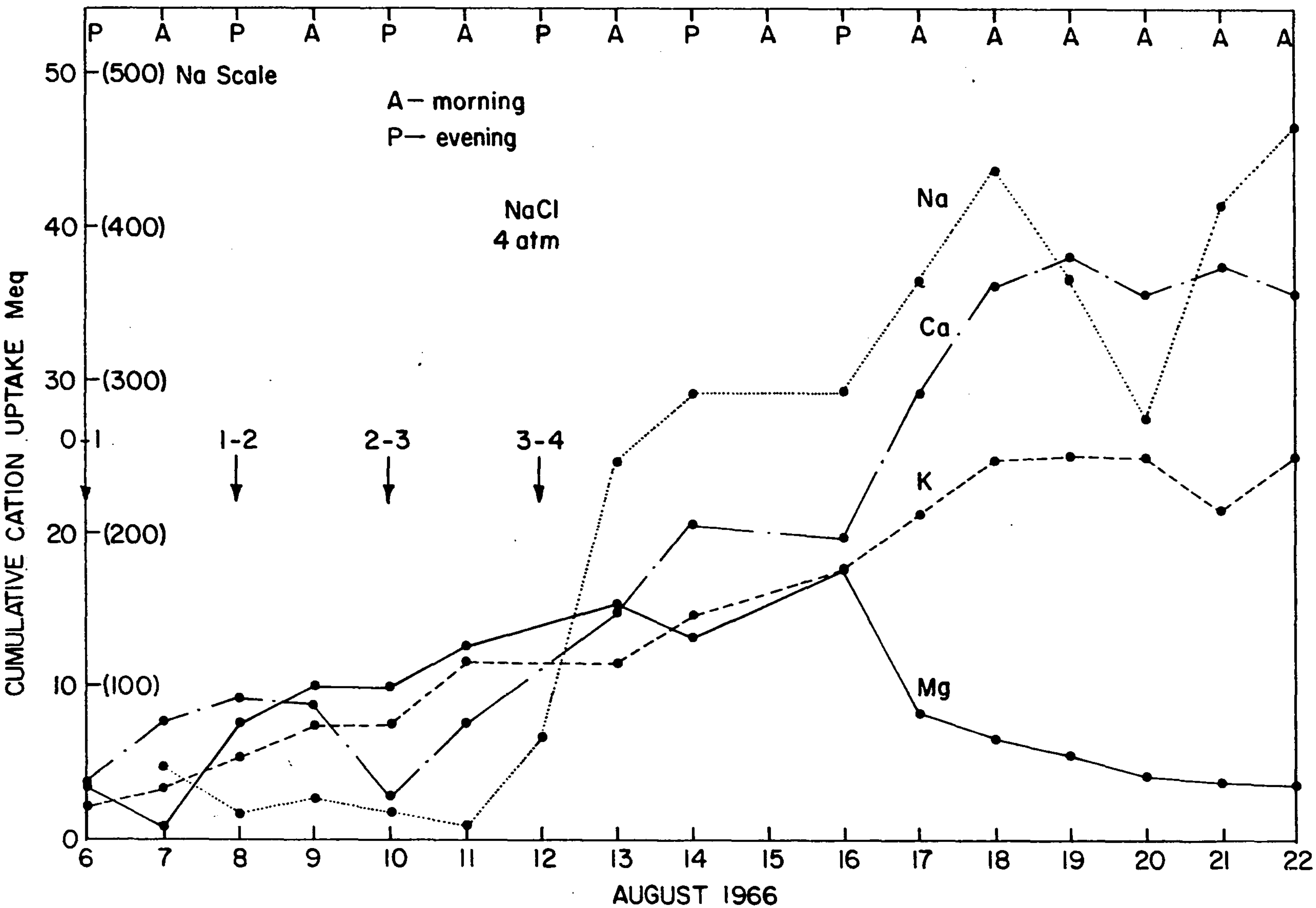

Fig. 15.--Cumulative Cation Uptake-Sodium Chloride. 4 atm Treatment

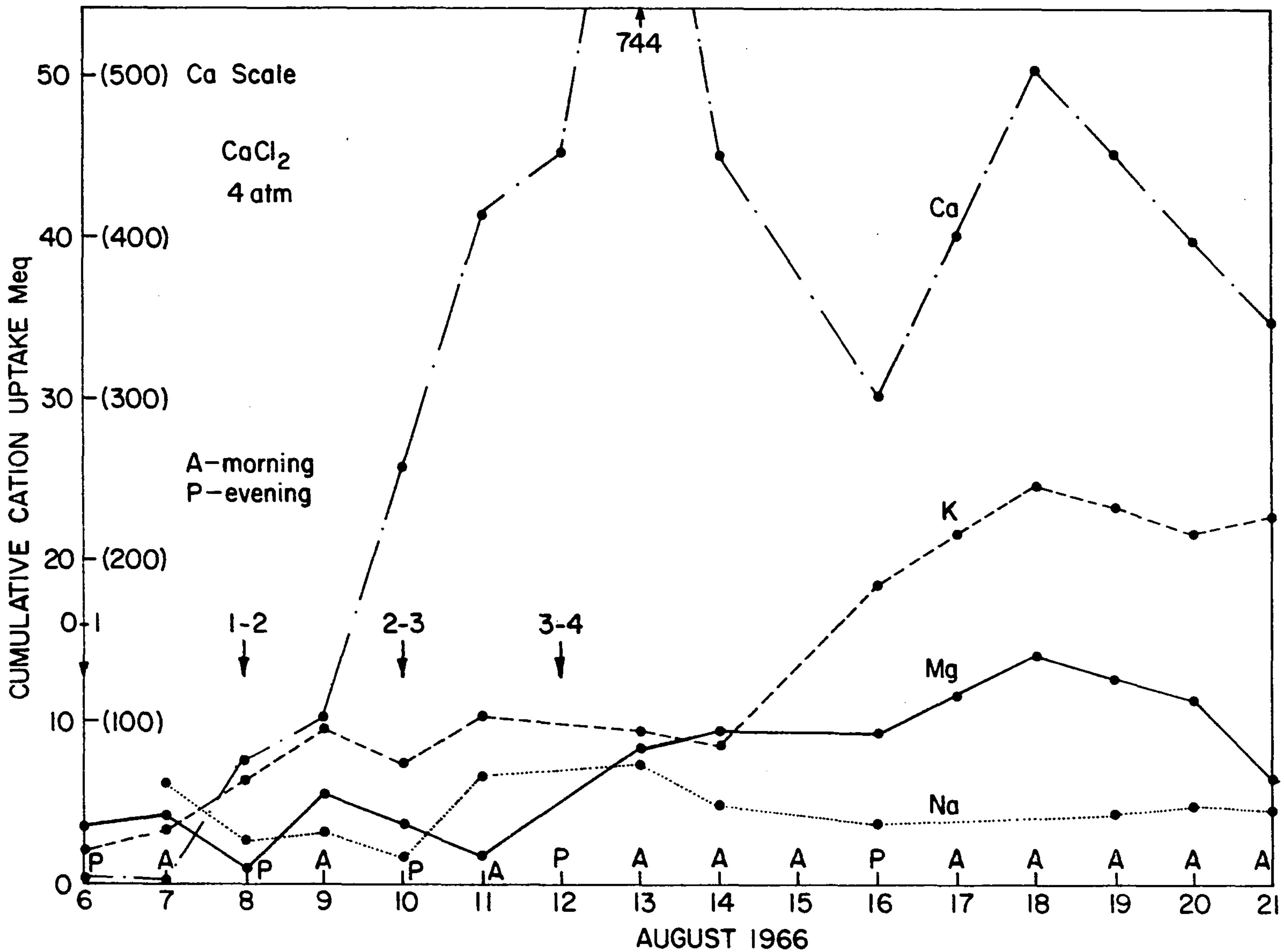

Fig. 16.--Cumulative Cation Uptake-Calcium Chloride. 4 atm Treatment

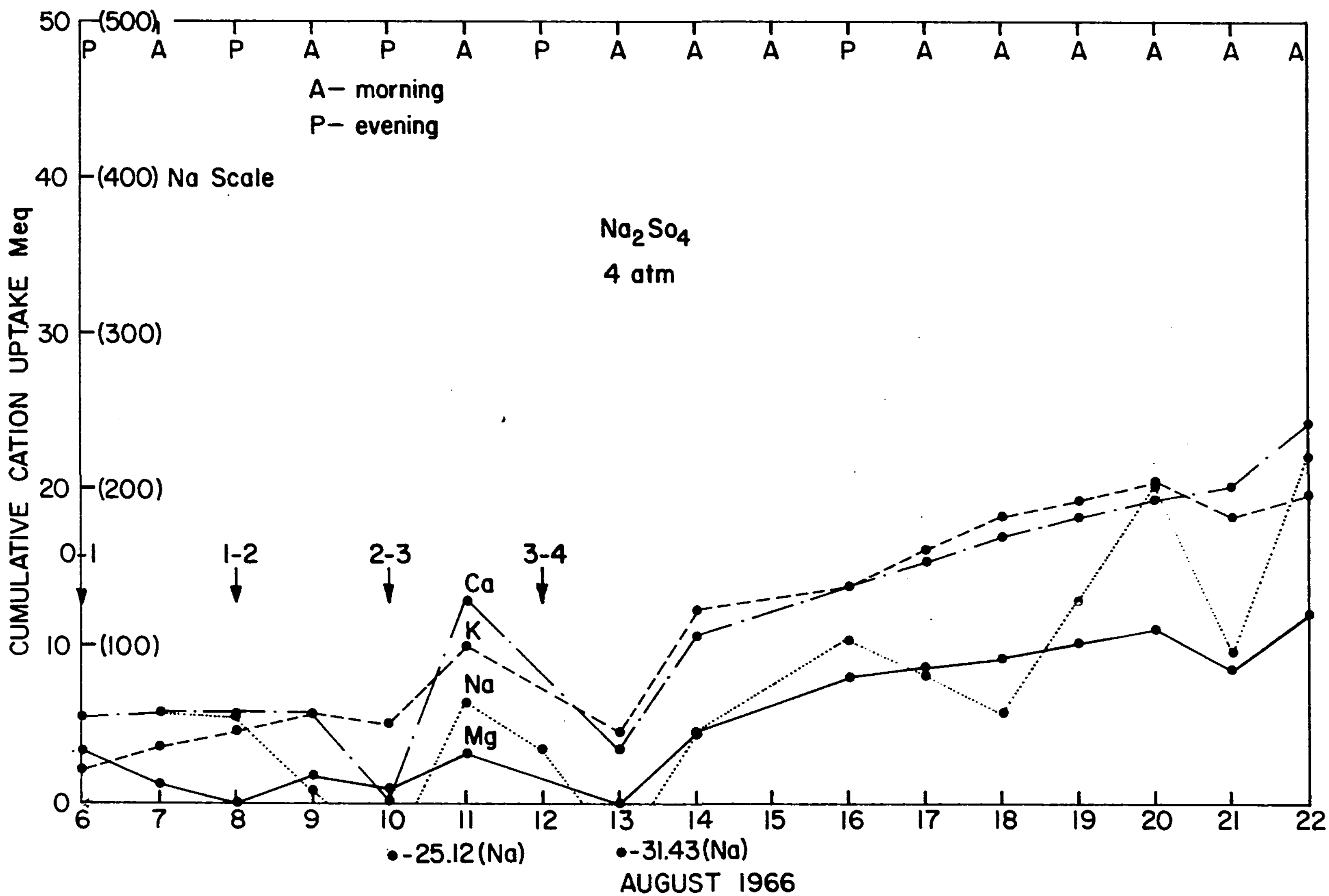

Fig. 17.--Cumulative Cation Uptake-Sodium Sulfate. 4 atm Treatment

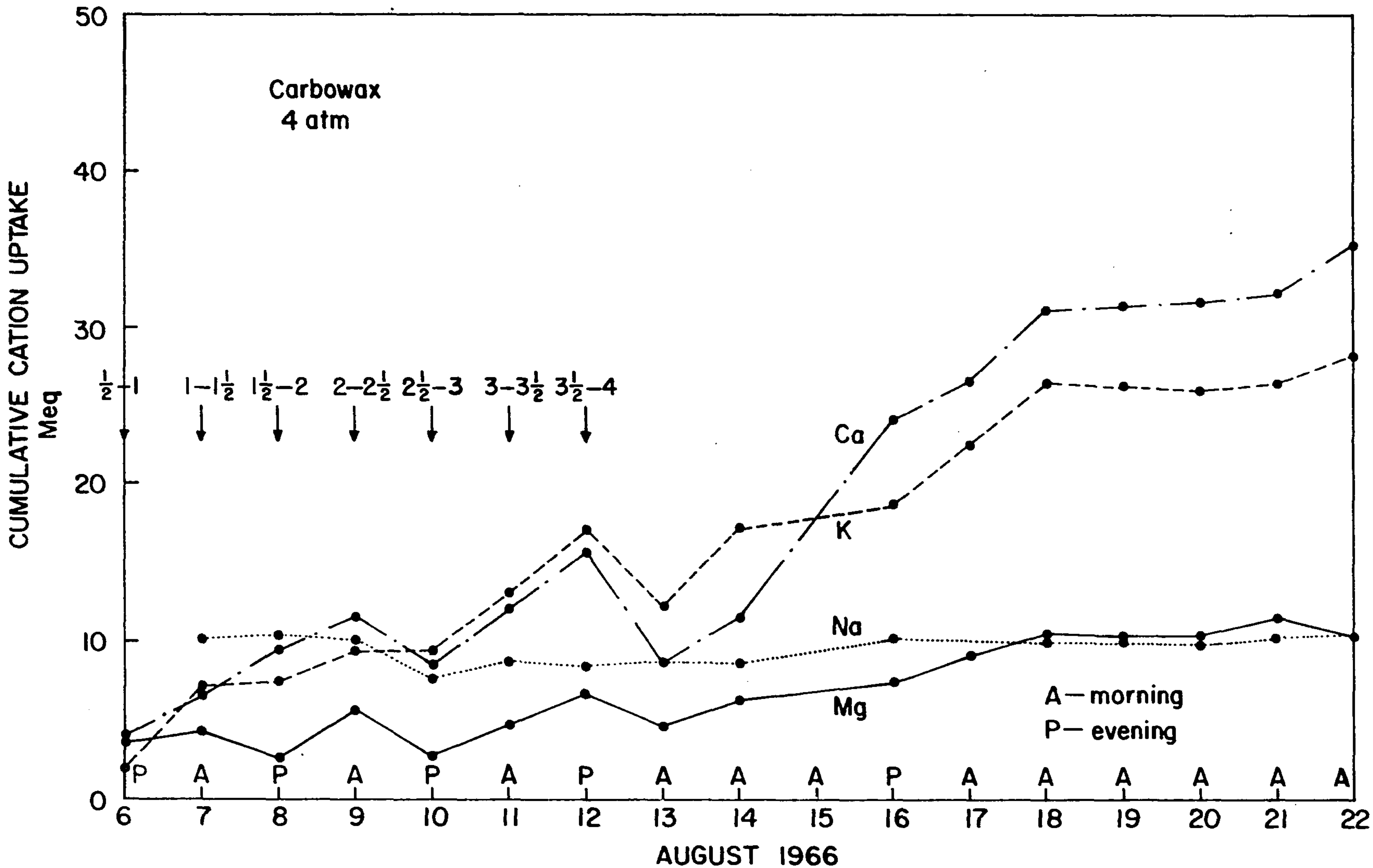

Fig. 18.--Cumulative Cation Uptake-Carbowax. 4 atm Treatment

uptake. Between 8/13 and 8/16, the plants lost more than half of the calcium previously taken up. When calcium was lost from the plants, accumulation of potassium began to increase. A slight increase in magnesium uptake occurred on the 16th through the 18th after which the plants lost magnesium.

The uptake of all ions from sodium sulfate enriched growth solutions was depressed. Final sodium uptake was about half that from sodium chloride treatments. Calcium uptake was the most suppressed while potassium and magnesium uptake were about the same as in the other salt treatments.

Carbowax treated plants withdrew ions from the growth solution in about the same proportion as the control plants but at a reduced rate. The decrease in ion uptake rate was most apparent during the last few days of the experimental period.

3.32 Summary of Uptake in Selected Periods. A summary of the ion uptake, expressed in milligrams, is given in Tables XXIV and XXV. The period from 8/6 to 8/12 represents the period osmotic additions were being made to bring the solutions up to their nominal treatment levels; the period from 8/13 through 8/16 represents the period with no osmotic additions prior to the nutrient change; and the period from 8/17 to 8/22 represents the period after the nutrient change when the solutions had no further osmotic additions and were assumed to be at their nominal osmotic potential levels of treatment.

TABLE XXIV

SUMMARY OF ION UPTAKE BY PLANTS
AT THE 2 ATM. LEVEL

mg/growth period

Time Interval	Treatment	Uptake in mg				
		Na	K	Ca	Mg	Cl
8/6—8/12	Control	2.99	581.81	374.75	97.28	0
	NaCl	1611.40	532.54	284.77	170.12	0 e
	$CaCl_2$	152.47	466.07	3899.79	76.60	221.33
	Na_2SO_4	796.39	489.53	199.20	36.73	0
	CBWX	194.55	363.24	334.67	69.44	0
8/13—8/16	Control	94.05	369.10	245.09	83.42	0
	NaCl	-324.72	119.65	119.44	0.25	0 e
	$CaCl_2$	-79.57	263.53	-321.04	65.91	2073.22
	Na_2SO_4	2041.67	291.69	200.80	46.21	0
	CBWX	28.52	376.53	188.38	70.53	0
8/17 - 8/22	Control	16.56	789.43	466.12	89.99	0
	NaCl	169.72	498.53	304.41	63.23	0 e
	$CaCl_2$	23.00	305.37	342.88	47.30	-734.23
	Na_2SO_4	-16.56	420.72	226.25	25.90	0
	CBWX	5.52	696.76	338.88	57.15	0

e - Exact uptake could not be calculated, but from data available, uptake appeared to be equal to zero.

Note: Chloride uptake in treatments, to which chloride was not added as a treatment, was assumed to be zero since the chloride content of the nutrient solution is negligible.

TABLE XXV

SUMMARY OF ION UPTAKE BY PLANTS AT THE 4 ATM. LEVEL

mg/growth period

Time Interval	Treatment	Uptake in mg Na	K	Ca	Mg	Cl
	Control	2.99	581.81	374.75	97.28	0
	NaCl	5711.99	459.82	298.60	186.29	4729.25
8/6—8/12	$CaCl_2$	172.48	374.58	14903.55	103.12	4794.50
(8/7—8/12) for Na	Na_2SO_4	-722.80	186.51	68.94	0.00	0
	CBWX	205.59	478.98	178.76	58.00	0
	Control	94.05	369.10	245.09	83.41	0
	NaCl	1013.71	226.39	100.40	27.97	939.26
8/13—8/16	$CaCl_2$	-84.17	356.59	-8844.65	7.90	-2621.69
	Na_2SO_4	3114.02	358.55	211.42	99.10	0
	CBWX	29.67	260.80	307.41	30.64	0
	Control**	16.56	789.43	466.13	89.98	0
	NaCl	4056.90	291.69	315.23	-167.69	-2162.52
8/17—8/22	$CaCl_2$*	14.95	161.87	897.39	-34.17	-1962.56
	Na_2SO_4	2769.99	235.77	210.82	49.73	0
	CBWX**	1.84	369.10	223.65	36.72	0

* Period 8/18 - 8/21 for Na. Period 8/17 - 8/21 for K, Ca, Mg and Cl

** Period 8/18 - 8/22 for Na.

Note: Chloride uptake in treatments, to which chloride was not added as a treatment, was assumed to be zero since the chloride content of the nutrient solution is negligible.

In the first period the sodium chloride treated plants took up about three times as much sodium as potassium at the 2 atm level; and more than ten times the potassium uptake at the 4 atm level. In the same period the plants at 2 atm took up no chloride while those at 4 atm took up almost as much chloride as sodium. In the period following the osmotic additions, the 2 atm treated plants lost about one-fifth of the sodium they had taken up while the plants at 4 atm continued to take up both sodium and chloride. In the final period the plants at 4 atm continued to take up sodium but they lost about one-third of the total chloride they had accumulated. The plants at 2 atm took up a small amount of sodium.

The calcium chloride treated plants accumulated eight times as much calcium as potassium at the 2 atm level and nearly forty times as much at the 4 atm level in the first period. The plants at 4 atm, in the same period, accumulated about the same amount of chloride as did the plants treated with sodium chloride. The 2 atm plants accumulated about half as much chloride as the 4 atm plants in the first period. In the period following the osmotic additions, both the 2 and 4 atm treatments lost calcium. The 4 atm treatments lost not only a greater absolute amount but also a greater amount in proportion to the total amount it had accumulated at that time. Chloride also was lost from the 4 atm plants in this period. But the 2 atm plants continued to accumulate chloride. In the final period, both levels of treatment accumulated calcium and lost chloride. The 2 atm treatments accumulated more calcium and lost less chloride than the 4 atm plants.

The uptake of all ions was much less in the sodium sulfate treatments than in other treatments. When uptake rates are depressed the accuracy of uptake calculations is decreased. Since uptake is measured as the rate of disappearance of ions from the nutrient solution, low uptake rates make the calculated uptake a small difference between large numbers. Hence in the first period the calculations show that the sodium sulfate plants at the 4 atm level lost more sodium than they accumulated during the experimental period. Evidently, the sodium uptake in this treatment, if any occurred, was very small for this period. At the 2 atm level the plants took up about twice as much sodium as potassium in the first period. In the period following the osmotic additions both levels of treatment took up sodium. In the final period the 4 atm treated plants continued to take up sodium while the 2 atm plants lost a slight amount of sodium.

Of the ions not added as part of a treatment, magnesium uptake was affected the most by the treatments. In the plants treated with sodium chloride, magnesium uptake was stimulated in the first period and depressed or rejected in the later periods. In calcium chloride treated plants, magnesium uptake followed the same pattern at the 4 atm level as the 4 atm sodium chloride treatment. Magnesium uptake was depressed in all periods for plants treated at the 2 atm level with calcium chloride. The plants treated with 2 atm of sodium sulfate had a similar constant depressed magnesium uptake, but at the 4 atm level, uptake was variable, ranging from zero in the first period to almost equal to the control in the second period to about one-half of the control in the final period. Plants treated with Carbowax showed a depression of potassium approximately in proportion to the level of the treatment.

The total net ion uptake for each treatment is shown in Table XXVI. The resultant uptake of sodium from the sodium chloride treatments was slightly greater than potassium uptake at the 2 atm level and more than ten times the potassium uptake at the 4 atm level. The other cations were depressed in proportion to the level of the treatment. There was no chloride uptake at the 2 atm level and about one-third as much chloride as sodium at the 4 atm level.

The uptake of calcium was only slightly higher for plants treated with calcium chloride at the 4 atm level than for plants treated at the 2 atm level. Uptake of potassium and magnesium was depressed in proportion to the level of treatment. The uptake of chloride at the 2 atm level was slightly greater than that taken up by plants at the 4 atm level of sodium chloride. There was only a slight net uptake of chloride from the 4 atm treatment.

Sodium sulfate treated plants took up twice as much sodium at the 2 atm level as did plants treated with sodium chloride. At the 4 atm level sodium uptake was twice that at the 2 atm level but only one-half

TABLE XXVI

TOTAL NET ION ACCUMULATION

milligrams/plant

Treatment	Level	Total Ion Uptake (milligrams)/Plant				
		Na	K	Ca	Mg	Cl
Control	---	114	1740	1086	271	0
NaCl	+2	1456	1151	709	234	0
	+4	10,782	978	714	47	3506
$CaCl_2$	+2	96	1035	5922	186	1561
	+4	103	893	6956	77	211
Na_2SO_4	+2	2822	1202	626	109	0
	+4	5161	781	491	148	0
CBWX	+2	229	1434	862	197	0
	+4	237	1111	710	125	0

that taken up by plants treated with 4 atm of sodium chloride. Potassium and calcium uptakes were depressed in proportion to the level of the treatments, but magnesium uptake was less at the 2 atm treatment than the 4 atm treatment.

At 2 atm of treatment with Carbowax, the uptake of all ions was reduced except for sodium. The uptake of sodium at the 4 atm level was slightly greater than at the 2 atm level. For the remaining ions the uptake at the 2 atm level was slightly greater than the uptake at the 4 atm level.

CHAPTER 4

DISCUSSION

4.1 Validity of the Premise

The premise on which this study was based is outlined in Figure 5. It is composed of results from several studies showing that plant growth is inhibited iso-osmotically (20), no specific toxicity exists for sodium or chloride (2), and plants grown in higher osmotic media maintain the gradient for water movement into the plant by osmotic adjustment of the plant sap (4). The validity of the components of the original premise are examined in the next subsections.

4.11 Iso-osmotic Growth Inhibition. The total plant dry weight expressed as a percentage of the control at each level of treatment for all the treatments is shown in Figure 19. The data presented by Gauch and Wadleigh (20) is also shown in Figure 19 for comparison. The total plant dry weight decreased with the treatment level for all the treatments, but not equally. The greatest difference between the treatments occurred at the 2 atm level of treatment. Sodium chloride and calcium chloride treated plants had the greatest growth depression at this level. Increasing the treatment level to 4 atm decreased plant growth by only a slight amount in these treatments. At the 4 atm

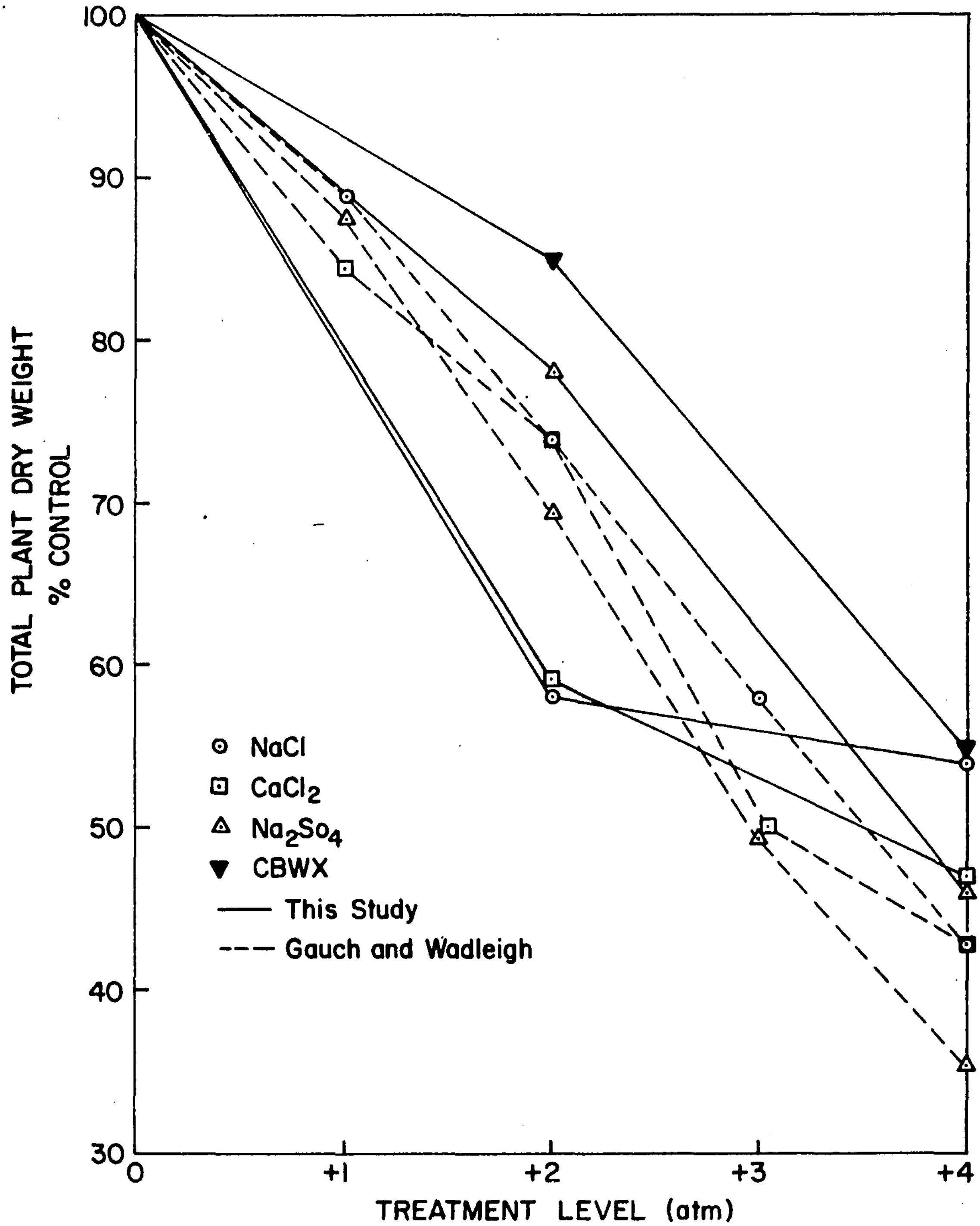

Fig. 19.--Red Kidney Bean Growth as a Function of Treatment Level. Comparison of Data from this Study to Gauch and Wadleigh (20) Data

level there was little difference between the treatments as indicated by the total plant dry weight, the leaf dry weight, and the leaf area per gram of leaf dry weight. Judging from these parameters, growth inhibition was iso-osmotic at the 4 atm level.

Carbowax 1540 was used as a treatment to distinguish osmotic effects from specific ion effects. The dry weight content of all the plant tissue (i. e. roots, leaves, stems and petioles) from the plants treated with Carbowax exceeded that for the salt treated plants. Similar results were obtained by Largerwerff and Ogata (38) in studies using Carbowax with a molecular weight of 15, 000-20, 000. The net assimilation rate of Carbowax treated plants and leaf initiation rate (at the 4 atm level) exceeded the corresponding rates for plants treated with one of the salts. The root tips of the plants treated with Carbowax showed the least change in morphology and most closely resembled the root tips of the control plants. However, the salt treated plants had higher values of leaf water content per gram of dry weight and more area per leaf than plants treated with Carbowax. In addition, at the 2 atm level, the leaf area per gram of dry weight was higher for salt than for Carbowax treatment.

Specific sodium ion effects probably are indicated when growth parameters show the same trend for plants treated with sodium chloride or sodium sulfate. The signs of sodium ion induced effects are most evident in the roots. Plants treated with sodium chloride or sodium sulfate had the lowest root dry weight at the 4 atm level and at both levels had the highest water content per gram of dry weight of roots. The sodium treated plants also had the highest respiration rates.

Specific chloride ion effects were most pronounced in the leaves. The effects were especially evident at the 2 atm level where the chloride treated plants had the lowest leaf dry weight and hence the highest area per dry weight of all the treatments including the control. At all levels of treatment, the chloride treated plants had the greatest leaf water content per unit of dry weight. Wadleigh and Ayers (74) found that chloride accumulation affected carbohydrate metabolism by possibly reducing photosynthetic activity. Our data show that the photosynthetic rate as indicated by the net assimilation rate was the lowest in the chloride treatments. The only root characteristic that was different for the chloride treated plants was at the 2 atm level. At this level the chloride treated plants had the lowest root dry weight.

It seems safe to conclude that iso-osmotic growth inhibition has been over stressed. There are recognizable specific ion effects sometimes in the form of sodium or chloride effect and sometimes, it seems, simply ion effects. Greater differences are more evident in the data from this study than in the data presented by Gauch and Wadleigh (20). One of the primary reasons is that the plants grown in our study attained a greater size. The control plants in our study had a dry weight of 39.6 grams and the control plants for Gauch and

Wadleigh's study reached a size of only 6.49 grams. Growth inhibition or specific ion effects will be more noticeable when the normal rate of growth is faster.

4.12 Osmotic Adjustment of Sap. Only plants treated with sodium chloride in this study can be said definitely to have made nearly complete osmotic adjustment of their leaf sap. Carbowax treated plants may have made some adjustment through accumulation of Carbowax. But the magnitude of this contribution is unknown. However, Janes (29) found the rate of entry of Carbowax extremely low even at the 14 atm level. Little or no adjustment of the osmotic gradient from the growth solution to the leaves was made by plants treated with sodium sulfate or calcium chloride. See Figure 10. However, previous studies showing osmotic adjustment always have used either sodium chloride or Carbowax (4), (5), (59).

Ion uptake data show several periods where univalent cations are accumulated and divalent cations lost. As has been mentioned, this is one of the proposed methods of osmotic adjustment (4). In the latter studies it always has been assumed that osmotic adjustment was synonomous with water potential adjustment. The data shown in Figure 12 show that this assumption is not valid. Carbowax treated plants were the only plants in which the water potential gradient was substantially maintained. Sodium sulfate treated plants made a partial adjustment of the water potential gradient, but sodium chloride and calcium chloride treated plants made little or no adjustment.

The data in Table X show that leaves treated with Carbowax or sodium sulfate had low water content per gram of dry weight. It seems likely that at least part of the adjustment of water potential in these treatments was by dehydration. Cell dehydration acts to increase the water potential (in a negative sense) by lowering the hydrostatic pressure potential in the cell and increasing the osmotic potential. A decrease in the pressure potential (or turgor pressure) would tend to inhibit leaf expansion. The data show that at the 2 atm level the leaf area per gram of leaf dry weight is the lowest for sodium sulfate and Carbowax treated plants.

An estimate of the pressure potential as compared to the control plants was made by combining the water potential and osmotic potential data. The results are shown in Table XXVII. Notice that since sodium chloride plants had nearly full osmotic potential adjustment, but little water potential adjustment, the pressure potential of these leaves exceeds the control. Calcium chloride leaves showed a slight increase in osmotic potential sufficient to cause their pressure potential to increase relative to the control leaves. When other factors are held constant an increase in the hydrostatic pressure within a cell, the pressure potential, should be reflected by an increase in cell expansion. The data in Table XI show that this in fact does occur at

TABLE XXVII

DEVIATION OF THE TREATED LEAF PRESSURE POTENTIAL FROM THE CONTROL (One Replicate)

atm

Date	+2 atm				+4 atm			
	NaCl	$CaCl_2$	Na_2SO_4	CBWX	NaCl	$CaCl_2$	Na_2SO_4	CBWX
8/7	0.2	-3.7	-1.2	-1.2	0.2	-3.7	-2.2	-1.2
8/9	2.9	1.8	-0.6	-0.4	2.9	1.8	-0.6	-0.4
8/11	2.0	-0.3	1.2	-0.8	1.4	-0.2	-0.5	-0.3
8/13	---	---	---	---	2.3	1.6	0.2	-0.9
8/21	3.7	1.3	-0.2	-0.8	4.1	2.5	0.1	-2.1
Mean	+2.2*	-0.2	-0.5	-0.8**	+2.2*	+0.5	-0.6	-0.4

* Significant at 5% level) One Tail "t" test paired data
** Significant at 1% level)

the 2 atm level for sodium chloride and calcium chloride. Although the calculated pressure potential was higher for the chloride treatments at the 4 atm level, the differences in leaf expansion were not as evident. This might be due to inhibition by salinity of other factors necessary for leaf expansion. Nieman (47) found that RNA and protein synthesis are decreased in bean leaves treated with sodium chloride.

4.2 Investigation of Initial Postulates

Based on the original premise, two possible explanations of salinity toxicity symptoms were put forward. The first was that symptoms of drought might be caused by an increase in the resistance of the plant tissue to water movement. The second pertained to symptoms of salinity toxicity resembling those of potassium deficiency. In this regard, it was postulated that perhaps some metabolic process is upset by the addition of salinity which is associated with a decrease in the potassium content of the plant. Data bearing on these two postulates are presented in the next two sub-sections.

4.21 Plant Resistance to Water Movement

4.211 Root Resistance. Data presented in Tables XIX and XX, and Figures 8 and 9 show that the resistance of the roots to passage of water was increased to some degree by all the treatments. Plants treated with the chloride salts showed the least increase in root resistance. Carbowax treated plants had the next highest resistance and plants treated with sodium sulfate had the highest root resistance. The fact that morphological changes were not as evident in the winter grown plants but resistance changes still occurred, seems to indicate that morphological changes are not entirely responsible for resistance changes.

Although the Carbowax treated roots most closely resembled the control roots in Figures 6 and 7, there is some evidence of secondary root initiation near the tip of the Carbowax treated roots. Jackson (28) presents data showing that Carbowax inhibits the elongation of root hairs. A similar effect on secondary roots might be expected. Hayward and Spurr (22) found that salinity inhibited root elongation which caused the area of maximum water uptake to be reduced and shifted nearer the root tip. Siemer (62) showed that in long term experiments, root tip elongation was inhibited in proportion to the level of salinity applied. Xylem development also was inhibited but not as much as elongation which resulted in the xylem being developed closer to the root tip. With cells maturing nearer the tip, one would expect the layer of suberin, which retards water entry, might extend further down the root and thus cut down water entry. In addition, one might expect that the initiation of secondary roots, requiring more mature tissue development also might occur nearer the root tip. Our data indicate that more secondary roots do develop near the end of the roots on the treated plants than on the control plants. Wright (79) reported that the primary roots of Bermuda seeds germinated under saline conditions were fat and short and stopped elongating a short time after emerging from the seed. They then developed adventitious roots between the seed coat and the stem. This appears to be similar to the development of adventitious roots in the treated plants in this study. The development of adventitious roots and the initiation of an increased number of secondary roots near the root tip resembles closely the tendency for stems to initiate more growing tips when the primary growing region is cut off or inhibited from growing.

When the morphological changes observed in this study are coupled with the observations of Nightingale and Farnham (50), indicating that roots in saline solutions become woody and lose their capability of protein synthesis, and the observations by Nieman and Willis (48) that protein was lost to the growth solution from saline treated roots, it seems evident that the morphological changes are associated with a disruption of protein synthesis. Webster (77) noted that pea roots' incorporation of amino acids to form proteins is increased by potassium and decreased by sodium. The results of El-Shourbagy's (14) study indicating that sodium growth inhibition could be partially overcome by the addition of a mixture of amino acids, also emphasized the tie between salinity and protein synthesis. It is not clear at this time whether the changes in root morphology are the cause or the effect of a decrease in protein synthesis.

4.212 Leaf Diffusion Resistance. The data in Table XXI show that leaf diffusion resistance is increased in all the treatments. Plants treated with Carbowax and sodium sulfate have the highest leaf resistance to vapor movement. The data in Table X show that the plants in these treatments also have the lowest leaf water content per unit dry weight. This is probably the result of the high root resistance in these treatments limiting the amount of water supplied to the leaves. Lowering the leaf water content probably reduces the leaf turgor. If the turgor of the guard cells around the stomata is lowered below the turgor for the adjacent epidermal cells, the guard cells will deflate and close the stomata (80). Since the leaves of plants treated with sodium sulfate and Carbowax have the highest leaf diffusion resistance, the stomata are probably at least partially closed on these leaves. However, the transpiration per unit leaf area, shown in Table XVIII was decreased only slightly for the Carbowax and sodium sulfate treatments. This shows how important both resistance and leaf water potential gradient are to water movement through the plant. Evidently the adjustment of the water potential gradient was sufficient to keep water moving through the plants at a relatively high rate even though the resistance to water movement in the plants was increased.

Plants treated with sodium chloride or calcium chloride showed a decrease in transpiration rate per unit leaf area. This is in agreement with data presented by Largerwerff and Eagle (40). In an earlier report Bernstein stated that transpiration rate per unit leaf area was "not particularly affected" by salinity (4). The data on which this statement is based do not permit either support or rejection of this conclusions (11), (13). From our data one would have to conclude that transpiration per leaf area is affected by salinity, and it is a function of the leaf diffusion resistance and the water potential gradient.

Data presented in Table XVII show that all the treatments have transpiration rates lower than the control. In addition, Figure 20 shows that the transpiration rate per unit total plant dry weight also is reduced by the treatments. This indicates that transpiration is reduced more than dry weight production.

4.22 Ion Uptake

4.221 Sodium-Potassium Competition. In previous studies, uptake of potassium has been shown to be inhibited by sodium (15), (16), (71). Our data, as shown in Figure 21, show that potassium uptake was lower than the potassium uptake of control plants in all the treatments, and that potassium uptake decreases as the level of treatments increases. All the salt treatments had less potassium taken up than in the Carbowax treatments, but there does not appear to be any specific sodium inhibition of potassium uptake. Earlier studies which have shown inhibition of potassium uptake by sodium have been conducted with excised roots (15), (16), (71). Some workers consider results

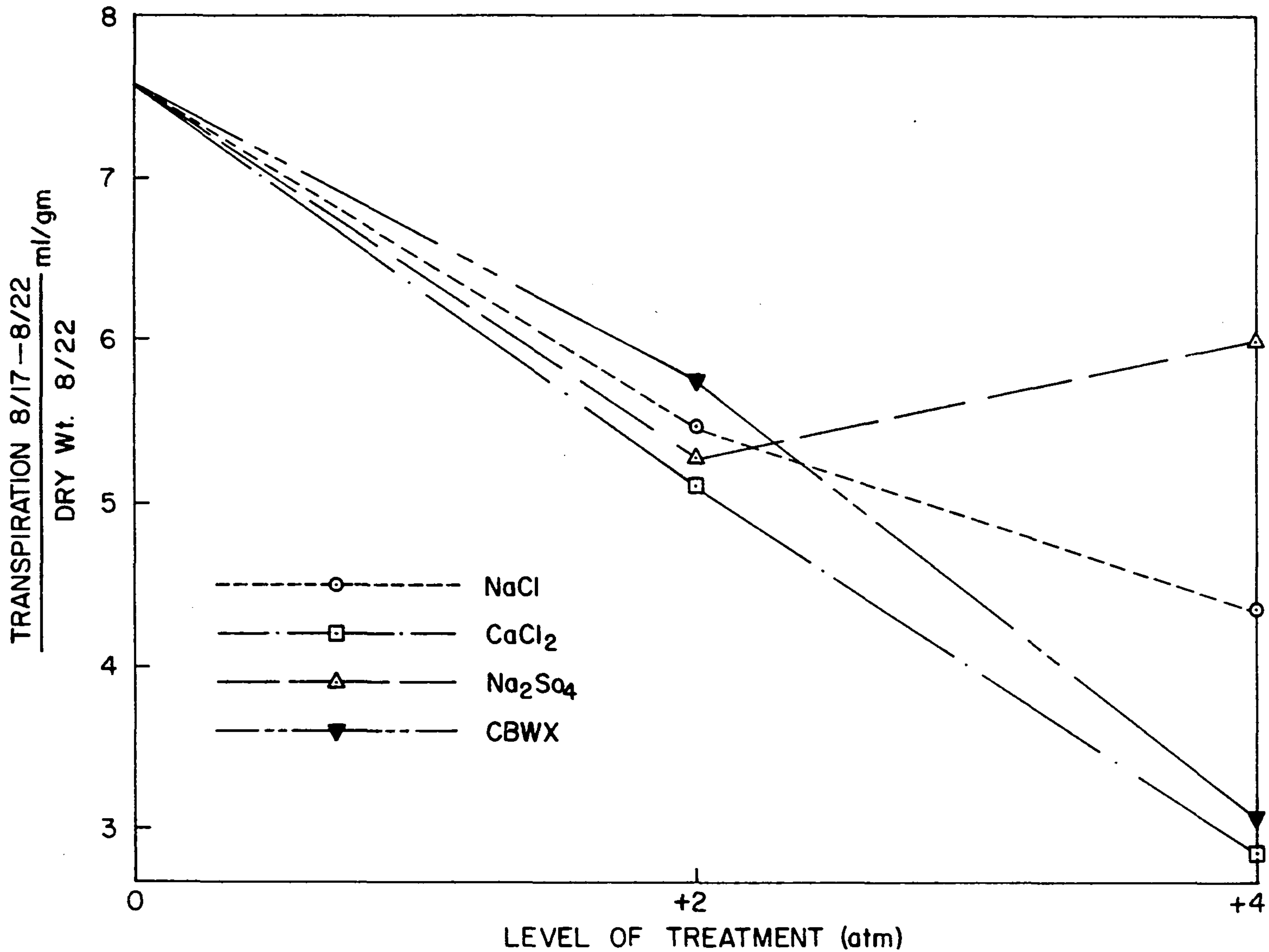

Fig. 20.--Ratio of Transpiration to Total Plant Dry Weight as a Function of the Treatment Level

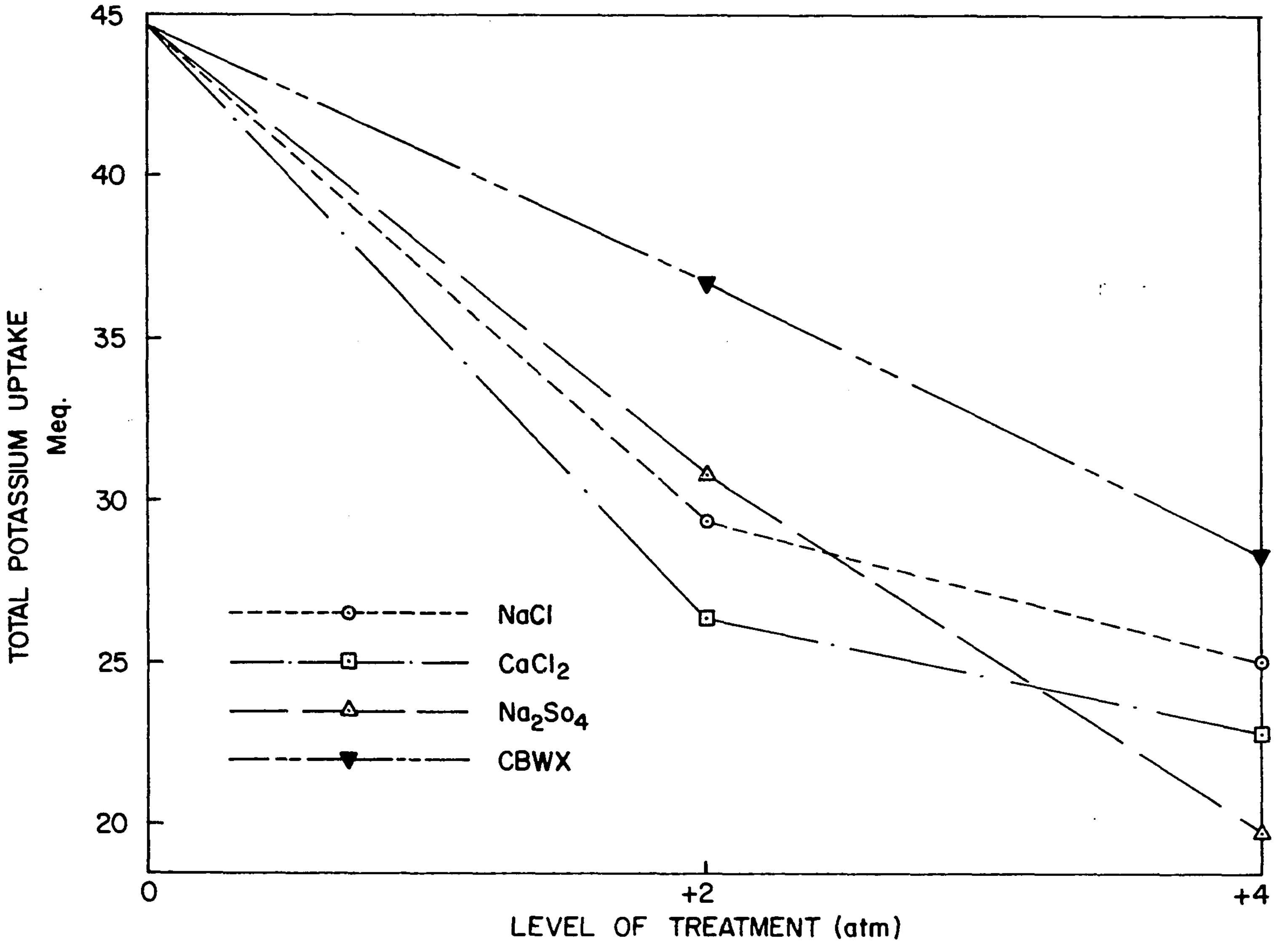

Fig. 21.--Potassium Uptake as a Function of Treatment Level

from such studies invalid because the real conditions existing in an intact plant are not simulated (65). The decrease in the potassium concentration accompanying the increase in sodium concentration in the roots as shown in Figure 4 does not necessarily mean potassium is rejected. Other studies have shown that many root systems have a high sodium storing capability (65). As the sodium concentration in the nutrient solution increases, the sodium uptake by the plant increases and the roots tend to store more sodium. Figure 21 shows that potassium uptake, under saline conditions, is decreased. The potassium content of the roots would decrease if upward potassium transport from the roots exceeds the rate of uptake of potassium by the roots.

The data presented from another study in Table IV showed that the ratio of potassium to sodium in the root sap decreased as the exterior concentration of sodium chloride increased. Our data, as shown in Table XXVIII, show that the ratio of potassium uptake to sodium uptake decreases for the treatments of high sodium ion concentration as does the ratio of potassium uptake to calcium uptake in the calcium chloride treatments. The ratios are shown for each treatment at the 4 atm level in Table XXVIII. For comparison, the ratio of potassium to sodium and calcium in the treatment solutions is shown in parentheses. Notice that although the ratio of potassium uptake to sodium or calcium uptake is reduced in the treatments in which these

TABLE XXVIII

RATIO OF POTASSIUM UPTAKE TO SODIUM AND CALCIUM UPTAKE (4 Atm.)

Meq/Meq

Time Interval	Treatment (+4)	Total K Uptake / Total Na Uptake	Total K Uptake / Total Ca Uptake
	Control	7. 81	0. 82
	NaCl	0. 053(. 041)	0. 70
8/6—8/22	$CaCl_2$	4. 16	0. 066(. 029)
	Na_2SO_4	0. 088(. 026)	0. 81
	CBWX	2. 79	0. 80

Note: Corresponding ratios of ions in nutrient solution are shown in parentheses.

ions were added the uptake ratio is greater than the corresponding concentration ratio in the solution. This indicates that potassium is still accumulated by the plant in a concentration exceeding that in the growth solution.

4.222 Cation-Chloride Uptake. The difference between the amount of cations accumulated and the amount of chloride ions accumulated has been postulated as giving an indication of the organic acid synthesis in the plant (69). When the difference in accumulation is positive, an increase in organic acid synthesis is indicated. The difference between the summation of the cations taken up by the plants and the chloride uptake, in Millequivalents, is shown in Table XXIX. If it is assumed that the organic acid synthesis for the control plants is normal, then departures from this normal synthesis would indicate changes in organic acid synthesis caused by the treatments. The differences are shown in Table XXX. The salt plants show a tendency to increase organic acid synthesis as the level of treatment increases and Carbowax plants show a decrease in organic acid synthesis as the level of treatment increases. Ulrich (69) found that organic acid synthesis actually preceded the excess accumulation of cations over anions. The data from our study shows that total cation accumulation is directly related to the estimated organic acid synthesis. However, no conclusive statement can be made since the estimate of organic acid synthesis involved using the summation of cations data. Thus organic acid

TABLE XXIX

DIFFERENCE BETWEEN CATION AND CHLORIDE UPTAKE

milliequivalents

Time Interval	Treatment	Level +2 atm	Level +4 atm
	Control	125.90	125.90
	NaCl	147.32	434.70
8/6—8/22	$CaCl_2$	297.42	359.60
	Na_2SO_4	193.63	281.15
	CBWX	105.90	84.45

TABLE XXX

DEVIATION OF THE DIFFERENCE BETWEEN CATION AND CHLORIDE UPTAKE FROM THE CONTROL

milliequivalents

Time Interval	Treatment	Level +2 atm	Level +4 atm
	Control	0	0
	NaCl	21.42	308.57
8/6—8/22	$CaCl_2$	171.52	233.70
	Na_2SO_4	67.73	155.25
	CBWX	-20.00	-41.48

synthesis, as computed by this method, is a dependent variable of the total cation accumulation, and it would be even more surprising if the two were not related.

4.223 Cation-Water Uptake. The amount of water removed from the growth solution decreased as the level of treatment increased. See Table XVII. The net uptake of ions, not added as part of a treatment, also decreased as the level of treatment increased. See Table XXVI. These data were combined to calculate the concentration of each ion in the water uptake stream. The Cation Concentration Factor or ratio of the concentration in the uptake stream to the concentration in the nutrient solution was calculated for each of the cations and is shown in Table XXXI. Cation Concentration Factors greater than one indicate that the concentration of the cation in the water uptake stream is greater than the concentration of that cation in the nutrient solution and thus selective uptake probably is indicated. At the 4 atm level there is a tendency for the Concentration Factors of monovalent cations to be raised and the divalent cations to be lowered relative to the control values. This is especially noticeable in the sodium chloride treatment where the sodium concentration factor is about four times the control value for sodium and about one-third the control value for Magnesium. The accumulation of monovalent cations at the expense of divalent cations is one method of osmotic adjustment. Since the sodium chloride treated plants were the only plants that were shown to adjust their sap

TABLE XXXI

CATION CONCENTRATION FACTOR IN THE WATER UPTAKE STREAM

ppm/ppm

Treatment	Level	Concentration Factor Na	K	Ca	Mg
Control		0.59	3.02	2.50	1.03
NaCl	+2	0.38	2.74	2.06	1.44
	+4	2.34	2.58	2.39	0.34
$CaCl_2$	+2	1.16	2.80	1.80	1.31
	+4	1.55	3.12	2.48	0.73
Na_2SO_4	+2	0.44	2.49	1.45	0.55
	+4	0.56	1.82	1.37	1.50
CBWX	+2	3.43	3.35	2.15	1.04
	+4	6.05	3.52	2.49	0.97

concentration, it seems likely that they did so by this method. The high sodium concentration factor for the 4 atm sodium chloride treated plants is especially noteworthy since the sodium level in the treatment solution is about thirty times that in the control solution. The Carbowax treated plants tended to have higher Cation Concentration Factors, except for magnesium. The Cation Concentration Factors for the ions in the sodium sulfate treated plants were the lowest of all the treatments, again with the exception of magnesium, which was the highest value for magnesium in all the treatments.

Although there are some trends in the variation of ion uptake as indicated by the Cation Concentration Factor; in general, the values for the treated plants are reasonably close to the control plant values. This indicates that over a rather large range of concentrations the amount of a cation taken up by a plant is nearly proportional to the concentration of the cation in the nutrient solution. There are different ratios between cations, but nearly constant ratios for any given cation over a wide range of concentrations. Thus the concentration of a cation in the water uptake stream would be constant if the concentration of the cation in the nutrient solution remained constant. Hence the governing factor for the amount of the cation taken up by the plant would be the amount of water taken up. Calculations were made of cation uptake for each treatment assuming that the amount of uptake for each cation was reduced below the control value the same amount the transpiration for that treatment was reduced below the control transpiration. The predicted cation uptake calculated in this manner is plotted against the actual cation uptake in Figure 22. The linear regression line is shown and, for comparison, the one-to-one relation line. The correlation coefficient is 0.98; therefore, about ninety-six percent $(0.98)^2$ of the variance between these two variables is explained by the regression equation. Only the uptake of cations not added as part of a treatment could be predicted by this technique so Figure 22 does not include the uptake of sodium in the sodium chloride or sodium sulfate treatments or the uptake of calcium in the calcium chloride treatments. The predicted uptake of other ions is slightly greater than the predicted uptake on the basis of water uptake alone. This difference becomes greater as the amount of water uptake increases.

From the net cation uptake data and the water content of the plants at the final harvest, the cation concentration per liter of water in the plant tissue was calculated. The results of the calculations for the control and 4 atm treated plants is shown in Table XXXII. The relationship between cation concentration in the plant closely reflect the uptake rates, as they should. Similar results were obtained when the ion concentration was expressed per gram of dry weight tissue. One will note that the potassium concentration is higher than the control in all the treatments except sodium chloride. In the latter the potassium

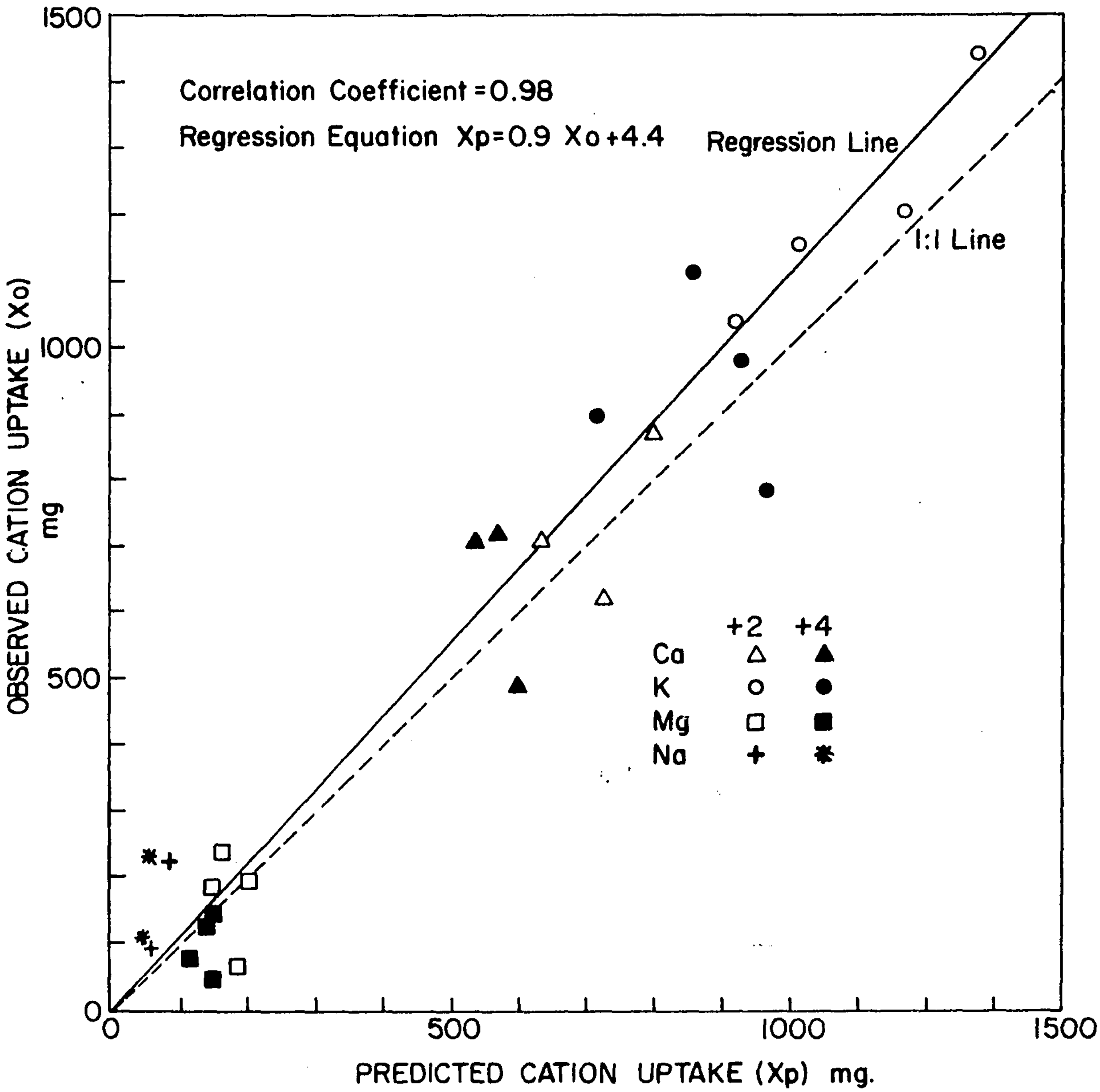

Fig. 22.--Predicted Cation Uptake Vs. Observed Cation Uptake

TABLE XXXII

TOTAL CATION UPTAKE PER LITER OF PLANT WATER CONTENT

meq / l

Time Interval	Treatment (+4)	Ion Uptake (meq)/Liter Water Content			
		Na	K	Ca	Mg
	Control	14.79	133.26	162.25	66.65
	NaCl	2392.19	127.60	181.84	19.54
8/6—8/22	$CaCl_2$	30.75	156.44	2377.53	43.29
	Na_2SO_4	1726.38	153.61	188.54	94.15
	CBWX	69.66	191.96	239.32	69.66

concentration is slightly lower than the potassium concentration of control plants. Thus, there does not appear to be any potassium deficiency. The only apparent deficiency is for magnesium in the sodium chloride treatment, and the data collected are not sufficient to indicate whether this deficit is enough to inhibit growth. The Carbowax treated plants have the highest concentration of nutrient ions.

4.3 Work for the Future

At present, even the most economic desalting plant can not produce pure water at a rate competitive with the current sources of water for conventional agriculture (68). The large volume of water required for field irrigation necessitates that the water be available at a low cost. Much of the water applied to a field is lost through infiltration or evaporation, and more than ninety percent of that which enters the plant is lost by transpiration. Large, closed enrionment greenhouses, such as described by Hodges and Kassander (25), in which the transpired water is caputred and re-used again, show promise for reducing the high water requirements for commercial agriculture. The environment in these houses is maintained at a high humidity which decreases the evaporative demand on the plants. Studies by Nieman (49) and Riley (57) have shown that plants irrigated with saline water show less growth inhibition in high humidity environments. Thus, research along this line shows promise for enabling brackish waters to be used for irrigation.

Environmental modification allows plants irrigated with saline water to survive with less growth inhibition because the requirement for water by the leaves is reduced. It does not, however, prevent the damage to the roots which causes a reduction in their water transmitting capability. Prevention of this damage must await further study of the plant's metabolic processes, particularly the protein synthesis in the roots under saline conditions. Insight into what processes are involved in causing the morphological and resistance changes in the roots may also be given by studying plants which have become adapted to salinity through successive breeding.

CHAPTER 5

CONCLUSIONS

The data collected in this study, as summarized in Figure 23, show that the premise on which the study was based was not quite accurate. That is, although growth and yield are decreased by salinity, the growth inhibition does not appear to be strictly iso-osmotic. The osmotic effects are the primary effects but specific ion effects can not be ignored. Plants treated with sodium chloride tend to adjust the osmotic potential of their leaf sap, but they do not increase their water potential sufficiently to maintain the gradient for water movement through the plant at the level observed in the control plants. Thus, adjustment of sap osmotic potential does not necessarily mean that physiological drought is avoided. Only sodium chloride treated plants made a significant adjustment of sap osmotic potential. However, plants treated with sodium sulfate and Carbowax 1540 made a partial adjustment of their total water potential. The unequal adjustment of osmotic and total water potential make it obviously clear that turgor, or pressure potential, observations must be made in future studies.

In addition to the decrease in the water potential gradient, the symptoms of drought caused by salinity are also induced by an increase in the resistance of roots to the passage of water. This is probably

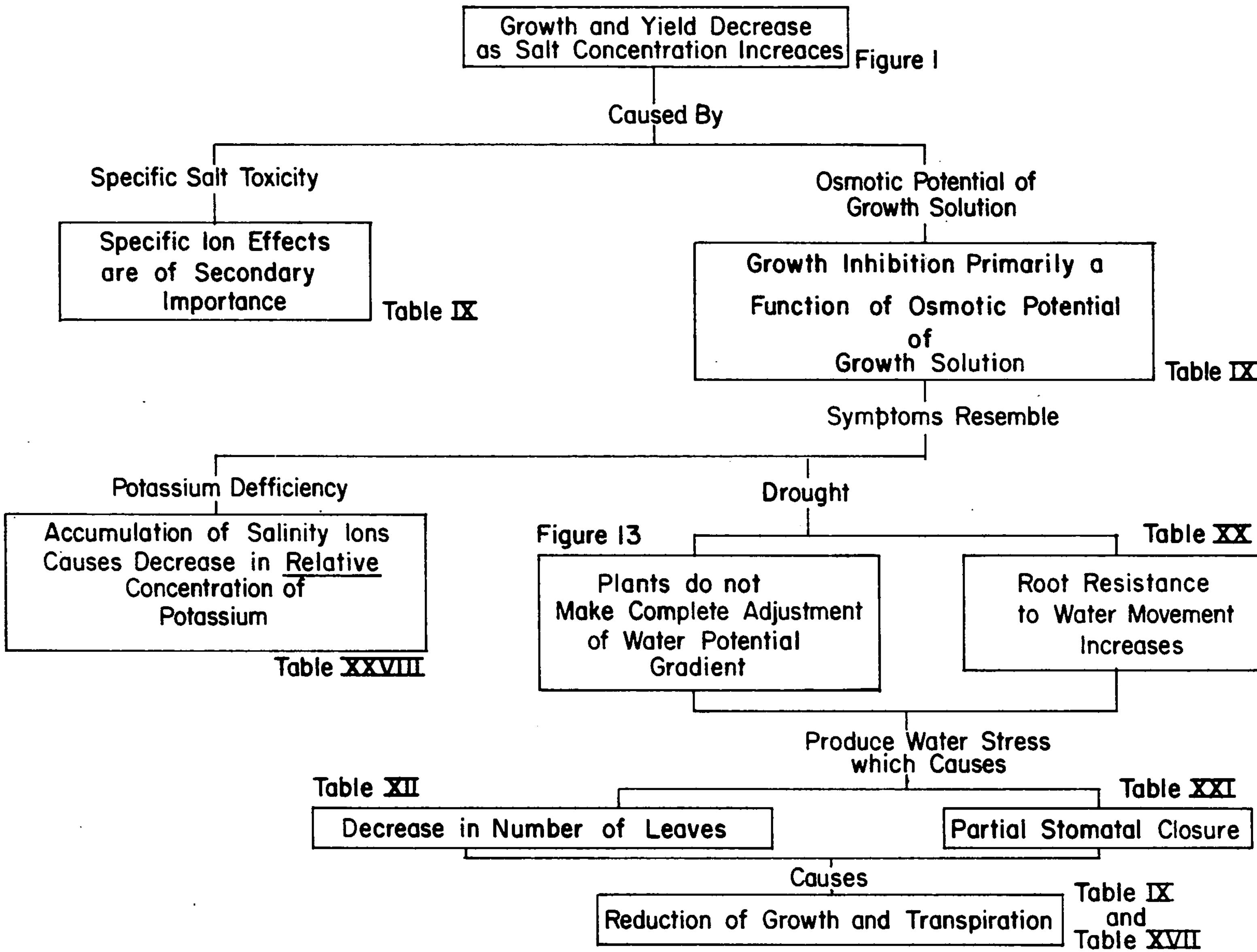

Fig. 23.--Summary of Salinity Research at the Conclusion of Study

the most significant physiological finding of this study and it is extremely important to the understanding of the effect salinity has on plant growth. The limitation of the water supply to the aerial portions of the plant produces a water stress. The plants under this stress have fewer leaves and the stomates on the leaves are partially closed. Both of these factors tend to reduce the water lost from the plant by transpiration and hence bring about a balance between the water lost from the leaves and the water supplied by the roots. As a result of the reduction in leaf area and the partial stomatal closure, the total photosynthesis of the salt treated plant is reduced. Thus, the plants under saline conditions have less production of dry matter and they develop the often reported stunted appearance.

Although no absolute deficiency of potassium is caused by high salinity concentrations, the relative concentration to other ions is decreased, and it is possible that this is responsible for the appearance of symptoms resembling potassium deficiency.

It appears that Aristotle was on the right track when he asked: "Why is it that celery can endure salt water, but the leek cannot? Is it because the roots of the latter are weak, but those of the former are strong, and that which is stronger is less liable to be affected?" (Aristotle, Book XX, Chapter on "Problems Concerning Shrubs and Vegetables")

APPENDIX

NUTRIENT SOLUTION PREPARATION

Compound	Concentration	Number of ml per 100 liters
KNO_3	1M	200
KH_2PO_4	1M	200
$MgSO_4$	1M	200
$Ca(NO_3)_2$	1M	300
Fe-EDTA	---	4.2 grams
Micronutrient concentrate*		100

*Micronutrient concentrate. Following added to 3 liters:

Compound	No. of Grams
H_3BO_3	7.50
$MnCl_2 . 4H_2O$	4.50
$CuCl_2 . 2H_2O$	0.15
MoO_3	0.15
$ZnSO_4$	0.66

ION CONCENTRATION IN NUTRIENT SOLUTION

Ion	Conc. ppm	Ion	Conc. ppm
Ca	120	Mn	0.416
NO_3	496	Cl	0.559
K	156.4	Cu	0.019
PO_4	190	Mo	0.334
Mg	48.6	Fe-EDTA	42
SO_4	192.1	Zn	0.089
BO_3	2.39		

OSMOTIC POTENTIAL OF CARBOWAX 1540
SOLUTION (44)

Osmotic Potential Bars	Grams of Carbowax/100 gm solution
1	4.75
2	7.50
3	9.75
4	11.90
5	13.80
6	15.40
7	16.70
8	18.00
9	19.20
10	20.25

CONCENTRATION OF SALTS AT TREATMENT
LEVELS OF OSMOTIC POTENTIAL

Osmotic Potential atm	NaCl Meq/l	$CaCl_2$ Meq/l	Na_2SO_4 Meq/l
1	23.8	33.7	35.0
2	48.5	68.0	72.6
3	78.2	102.5	111.7
4	98.2	137.6	151.8

OSMOTIC POTENTIAL VS. CONDUCTIVITY DATA

Solution	Regression Equation	Correlation Coefficient	Standard Error of Estimate atm
Nutrient Solution (N.S.)	$OP = 3.21C \times 10^{-4} - .10$	.988	.020
N.S. + NaCl	$OP = 3.98C \times 10^{-4} - .22$	.966	.044
N.S. + $CaCl_2$	$OP = 3.00C \times 10^{-4} - .09$	.968	.202
N.S. + Na_2SO_4	$OP = 3.22C \times 10^{-4} - .09$	.985	.119

OP = Osmotic Potential (atm)
C = Conductivity (micromhos)

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