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GROWTH AND NUTRITION OF PLANTS AS AFFECTED BY DIFFERENT  
OSMOTIC CONCENTRATIONS OF CALCIUM CHLORIDE AND  
SODIUM CHLORIDE IN THE SUBSTRATE

by

Muntaz Ali Khan

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Irrigation and Soils

UTAH STATE AGRICULTURAL COLLEGE  
Logan, Utah

1956

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Thanks are also due to Dr. Rex L. Hurst for his help in the statistical analysis of the results.

Mumtaz Ali Khan

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## INTRODUCTION

Considerable experimental evidence supports the concept that the growth of plants generally decreases progressively as the salt concentration of the substrate increases, but certain relationships between plant and substrate are still not fully understood. The chemistry of salt toxicity to plants involves many interactions both as to the quantity and kind of ions presented to the roots and those accumulated in the plants. Many plant species have shown sensitivity to excess accumulation of specific salts frequently encountered in saline soils. Thus Eaton (1942), Wadleigh, Hayward, and Ayers (1951) have shown most of the fruit trees to be susceptible to injury as a result of the accumulation of chloride ion. Wadleigh, et al. (1951) have reported orchard grass to be sensitive to calcium salts. Recently, Brown, Wadleigh, and Hayward (1953) have found calcium chloride more toxic to some fruit trees than isosmotic levels of sodium chloride. These and other studies have indicated a greater influence of specific ions than of the osmotic pressure of the solution.

The failure of crops in a given saline soil may be due to a variety of factors. Moreover, the relative importance of the contributing factors may be difficult to assess. For example, the deleterious effects of osmotic pressure per se or of individual elements are unknown. Also, the relative importance of high concentration of calcium salts, or the inaccessibility of essential elements induced by the presence of sodium, is far from being quantitatively related to growth inhibition observed in random situations.



The present system of salt tolerance evaluation of crops is based on the use of equal proportion of calcium chloride and sodium chloride in artificially salanized plots. This basis is, however, open to question for the real evaluation of calcium-sensitive plants. Specific sensitivity to chloride and calcium is being emphasized inasmuch as previous investigations have indicated the possibility that poor salt tolerances of some legumes and cereals may be related to accumulation of those ions in the plant tissues.

In view of the extreme importance of these questions in connection with the proper evaluation of salt tolerance of some of the important forage plants, the research here reported was devoted to the evaluation of some of these contributing factors as single entities.

A quantitative study was made of the specific effects of high concentrations of calcium chloride and sodium chloride on ionic absorption by the plants adequately supplied with nutrients to make consistently satisfactory growth.

## REVIEW OF LITERATURE

Salt accumulation in saline soil is usually a mixture of the components of several salts, but in certain areas a given cation or anion may predominate. According to the report of National Resources Planning Board (1942), in many regions subject to salinization, sodium is the main cation found in soil solution; in other regions calcium, or more rarely magnesium, is preponderant. The anions present in such soil solutions are frequently found to be mostly chloride, but sulphate ion may also be present in excessive amount. Even nitrate ions occasionally accumulate. Magistad and Reitemeier (1943) have shown that variation in the proportion of these ions may be practically infinite.

From the point of view of plant growth on saline soils, excessive salts dissolved in the soil solution assume immediate importance. It is here that the significance of salt concentrations, per se, can best be understood, since it is obvious that there must be some physiological limit to the plant's ability to absorb water from solutions of high osmotic pressure. Although physical factors such as capillarity ordinarily dominate the total water stress in soil (Veihmeyer, 1950), the osmotic pressure of the soil solution proper is additive. Correlation between high salt concentrations of the soil solution and unsatisfactory growth of agricultural crops have been shown by many, including Magistad, et al. (1943), Reitemeier (1943), Hayward and Spurr (1943), and Gauch and Wadleigh, (1944, 1945).

Magistad and Christiansen (1944) obtained a linear relationship between salt concentration and the growth of alfalfa. Similar linear

relations have been demonstrated for most other crops tested (Wadleigh and Ayers, 1945, and Gauch and Wadleigh, 1948). Salt tolerant crops have a slightly sloping regression line for decreased yield with increasing osmotic pressure. Salt sensitive crops have a steeply sloping line. In studies at the U. S. Salinity Laboratory, salt concentrations sufficient to cause osmotic pressures in excess of 7.7 atmospheres have not been used. Extrapolation of the regression lines for sugar beets and milo indicates, however, that growth of these crops would cease at about 10 to 12 atmospheres (Thorne and Peterson, 1954). Since the permanent wilting point of plants is not usually reached until a total water stress of about 15 atmospheres is attained, there is evidence that there are other effects of salts which limit crop growth in addition to the relations to water absorption.

Whereas much evidence points to osmotic pressure as a factor in plant nutrition, other effects of salts may be equally important in restricting the growth of certain species. Depending on the species, each of the various components that may be present in saline solutions may have some specific toxic effect on the plant over and above that which may be accounted for on the basis of the osmotic pressure of the soil solution. The influence of excessive concentration of specific salts on plant growth is an extremely complex subject involving many fundamental principles of plant nutrition. Much of the pertinent literature is cited in a review by Hayward and Wadleigh (1949). The literature citations in the following discussion will be restricted mainly to papers of special significance regarding sodium ( $\text{Na}^+$ ) and calcium ( $\text{Ca}^{++}$ ) ions only.

#### Effects of sodium salts on the growth and nutrition of plants

There is relatively little evidence that indicates positively the

specific toxicity of the  $\text{Na}^+$  to plants growing in saline soils. Many species tend to exclude sodium (Collander, 1941; Gauch and Wadleigh, 1945; Hayward, et al., 1946; and Wallace, et al., 1948), and specific toxic effects may arise from such exclusion of sodium along with accumulation of accompanying anions from the substrate as indicated by Hayward, et al. (1946). Notwithstanding this extreme selectivity in accumulation of sodium by plants, a few well-defined instances of sodium toxicity have been observed by Lileland, et al. (1945)--as tip burn of almond leaves which was related to sodium contents--and Ayers and associates (1951) have described a sodium scorch of avocado leaves. In both studies, the soils on which affected trees grew were sufficiently low in soluble salts and exchangeable sodium to be regarded as non-saline and non-alkali.

Although sodium salts in water cultures rarely cause toxic plant reactions, repeated tests in which sodium chloride has been compared with calcium chloride by Hayward and Long (1943), Hayward and Spurr (1944), Gauch and Wadleigh (1942, 1944, 1945), and Magistad (1945) have failed to show that it is unduly toxic in sand and solution cultures at isosmotic concentrations. But recently, Brown and others (1953) in the case of stone fruit trees and Ayers (1950) in the case of avocado evidenced the same types of leaf injury in sand or water cultures containing added sodium salts as were observed in the field, thus confirming the relationship of sodium to leaf injury in these species.

In spite of the above facts, Lehr (1942-1944) attributes the stimulative effect of sodium on sugar beets to the fact that sodium effectively counteracts absorption of calcium, thereby preventing the development of what he calls a "calcium-type plant." Recently a considerable amount of literature has accumulated showing beneficial

results from soil treatment with smaller quantities of sodium salts than would be considered significant in saline soils. (Chilean Nitrate Education Bureau, 1948)

The  $\text{Na}^+$  can apparently substitute to some extent for potassium ion ( $\text{K}^+$ ) in the normal growth of certain plants. As in the case of anions, it is difficult to interpret the effects produced by soluble  $\text{Na}^+$ , since both cations and anions may be involved simultaneously. The observed effect may have been caused as much by the one as the other. There is very meager information available regarding effects of sodium on the uptake of other nutrients of plants. Lehr (1941) and Harmer and Benne (1945) postulate the alteration in ratios of the various ions present when a sodium salt is added to the culture medium may also have something to do with the effects on growth. Wadleigh and Bower (1950) found that  $\text{Na}^+$  in solution is not actively taken up by bean plants. Also, the addition of 24 me./liter of  $\text{NaCl}$  to the basic culture solution caused practically no alteration in the calcium content of the dry matter of bean plants. However, the amount of growth was substantially reduced. Bower and Wadleigh (1948) found that beet plants and Rhodes grass absorbed much more  $\text{Na}^+$  in relation to  $\text{Ca}^{++}$  and magnesium ions ( $\text{Mg}^{++}$ ) than beans and Dallis grass. It is also pertinent to point out that in a brief absorption period Jenny and Overstreet (1939) found that exchangeable  $\text{Na}^+$  is more actively absorbed by barley roots than  $\text{Na}^+$  in solution as  $\text{NaCl}$ . Further research of Overstreet, reported by Kelley (1951), indicates that soluble sodium has a marked effect on the absorption of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{K}^+$  by barley roots.

#### Effects of calcium salts on the growth and nutrition of plants

The  $\text{Ca}^{++}$  may accumulate to high concentration in saline soil solutions, and this concentration may be specifically toxic. The specific

effect of high concentration of  $\text{Ca}^{++}$  varies with the species. For example, Wadleigh and Gauch (1944) found guayule to be relatively more tolerant of a saline substrate induced by  $\text{CaCl}_2$  than to those induced by other neutral salts. While on the other hand, early experiments at the California Experiment Station (1921) indicated  $\text{CaCl}_2$  is more toxic to barley than  $\text{NaCl}$  when compared on an equal osmotic basis. Masaewa (1936) found that applications of  $\text{CaCl}_2$  to soil cultures of flax were more highly toxic than applications of  $\text{NaCl}$ . She reported greater accumulation of chloride ions ( $\text{Cl}^-$ ) in plants on  $\text{CaCl}_2$  culture and attributed toxicity partly to chloride accumulation and partly to an unfavorable Ca/K ratio. Similarly, Wadleigh, et al. (1951) have shown orchard grass to be sensitive to calcium salts, but they also noticed that salinization of the soil with  $\text{Ca}(\text{NO}_3)_2$  produced the same effect as  $\text{CaCl}_2$ . Hence  $\text{Cl}^-$  toxicity was not involved. Bernstein and Ayers (1951) have secured comparable data for tall fescue grass and for bean plants.

More recent findings of Brown, et al. (1953) with stone fruit trees suggest a different type of toxicity induced by  $\text{CaCl}_2$ . They maintain that toxic effect in this case had resulted from increased  $\text{Cl}^-$  accumulation in the presence of high concentration of soluble calcium in the substrate. They further indicated that excess calcium uptake from substrates high in calcium was not an important factor in the development of injury due to these treatments.

Newton's (1923) studies with barley and peas showed little difference in the calcium and potassium content when these plants were grown in culture solution with high electrolyte concentration. However, the calcium in peas was 2.3 times higher than that in barley when these two plant species were grown in soil. Drake, et al. (1951) from his

studies concluded that roots with high cation exchange capacity, such as alfalfa, ladino clover, and head lettuce, absorbed adequate amounts of potassium. According to Mattson's (1948) theory, with an increase of free electrolytes, the inequalities of the Donnan distribution of ions is evened out in the plants. Other researches will be referred to in the discussion of observation and results.

From the above studies, involving only broad principles and giving discordant results, no definite conclusions are to be drawn as to the influence of specific salt present in the substrate upon plant growth. It is true, that the specific effect of excess accumulations of a given saline in the substrate upon plants has not been adequately established. By virtue of their differential effect on lowering the activity of the water and influencing the protoplasm of the plant, they demand elaborate investigation individually. But, unfortunately, very meager information exists, and there is need for more clarification of the specific effects of excessive concentrations of sodium chloride and calcium chloride.

Accordingly, an experiment was set up in which different levels of NaCl and CaCl<sub>2</sub> individually and in various combinations were added to a basic nutrient solution on barley and Hubam clover.

## EXPERIMENTAL PROCEDURE

The experiments reported herein were conducted during the summer of 1954 in the greenhouse at Utah State Agricultural College, Logan, Utah. Hubam clover and barley were selected in preference to other leading crops because of their wide geographic adaptation and adaptability to saline conditions. To avoid complicating soil conditions, the effects of various added salts on the growth of plants were carried out in solution cultures.

The experiments in the present investigation were laid out in a completely randomized design, as suggested by Cochran and Cox (1950), for the purpose of evaluating simultaneously the specific effects of interrelated factors. The treatments were as follows:

1. control (Hoagland's nutrient solution)
2. control + 100:0 mixture of NaCl and CaCl<sub>2</sub>
3. control + 75:25 mixture of NaCl and CaCl<sub>2</sub>
4. control + 50:50 mixture of NaCl and CaCl<sub>2</sub>
5. control + 25:75 mixture of NaCl and CaCl<sub>2</sub>
6. control + 0:100 mixture of NaCl and CaCl<sub>2</sub>.

The effect of treatments from 2 to 6 were studied at four levels of 1, 3, 6, and 9 atmospheres, osmotic pressure. Each treatment was replicated thrice in a total of 126 plots.

Hoagland's nutrient solution used in these experiments contained the following nutrients:

KH <sub>2</sub> PO <sub>4</sub>	1 me./liter
KNO <sub>3</sub>	5 me./liter



$\text{Ca}(\text{NO}_3)_2$	10 me./liter
$\text{MgSO}_4$	4 me./liter

A mixture of minor elements was added to give 0.5 p.p.m. boron, 9.5 p.p.m. manganese, 0.05 p.p.m. zinc, 0.02 p.p.m. copper, 0.01 p.p.m. molybdenum, and 0.02 p.p.m. iron. The above component salts were added to Logan tap water and desired pH of 6.0 was maintained throughout the experiment by 0.1 N  $\text{HNO}_3$  or with the hydroxide of the dominant cation. The container held 3.5 liters of solution which was constantly aerated.

By adding various amounts of  $\text{NaCl}$  and  $\text{CaCl}_2$  alone and in various combinations to the base nutrient solution, the relationship between osmotic concentration and specific electrical conductance was determined so that frequent periodic determinations of concentration could be made by conductance measurements.

Seven-day-old seedlings of barley and ten-day-old seedlings of Hubam clover were transferred to the basic nutrient solutions. One week after the seedlings were transplanted to the nutrient, those which were to receive added salts were given the initial salt increment (an amount sufficient to raise the osmotic concentration of the solution 1 atmosphere). Cultures which were to have more than one increment were given sufficient amounts to increase the osmotic concentration 1 atmosphere each day until the desired level was reached. This technique was adopted to permit better adjustment of the plants to the increased osmotic pressure of the nutrient solution.

*? How could one tell by conductance whether a given nutrient concentration was used and whether or not this was a limiting factor*

Table 1 lists the salts studied and the number of me./liter of each at the various osmotic concentrations. The volume of solution and its pH and concentration as regarding required osmotic pressure were periodically checked at an interval of 48 hours with necessary adjustments in order to make a uniform solution available to the root

Table 1. Milliequivalents of salt added to each liter of basal nutrient solution\* to give total osmotic concentrations of 1, 3, 6, and 9 atmospheres

Salt	Total atmospheres osmotic concentration			
	1.0	3.0	6.0	9.0
NaCl : CaCl <sub>2</sub>				
100 : 0	6.6	65.7	128.5	228.5
75 : 25	7.1	71.5	134.5	240.0
50 : 50	7.5	75.0	147.5	252.0
25 : 75	7.8	80.5	160.0	280.0
0 : 100	8.0	87.0	172.0	305.0

\* Osmotic concentration of basal nutrient solution, 0.67 atmospheres.

system. Temperature of the greenhouse was maintained between 25°-30° C. throughout the experiments.

Plant sampling and chemical analysis procedure

*Plants*  
The experiment on barley was started on July 19, 1954, and plants were harvested on September 8, when the plants headed out in most of the pots. Similarly, experiment on Hubam clover was started on August 6, 1954, and plants were harvested on September 29, when the first flower buds were beginning to open in most of the pots. After removing surface contamination of the plant material by brushing and brief rinsing of roots in distilled water, the plants were divided into (a) leaves and stem and (b) roots.

The samples were dried rapidly in a forced-draft oven at 70° C., the dry weight was obtained, and the samples ground in a small Wiley mill. A weighed portion of the sample was wet ashed with a mixture of nitric and perchloric acids. The concentration of sodium and potassium in the digest was determined by the use of the flame electrophotometer. Calcium and magnesium were determined by the versenate method as described by the Hach Chemical Company (1951). Interfering ions were removed by the method of Cheng, et al. (1953). Phosphorous was determined in the digest by the photoelectric colorimeter. A modification of the method described by Clark, et al. (1942) was used to determine chloride.

## RESULTS AND DISCUSSION

Experiment on barley

At the time differential treatments were started, all barley plants were approximately of the same size and initially made luxuriant growth; but, after a fortnight, differences in growth were apparent and leaf symptoms had developed on the plants in the jars with increasing  $\text{CaCl}_2$  treatment. The first observable effect of the salt treatment was an incipient wilting of basal leaves which was most pronounced under  $\text{CaCl}_2$  treatment. This was followed by tipburn of the leaves which became increasingly severe with time and resulted in death in some cases, especially at the highest concentration before the conclusion of the experiment. The condition of plants after two weeks of treatments is illustrated in figure 1. The plants were harvested after another three weeks of treatment. It may be pointed out that in the  $\text{NaCl}$  series, some of the plants did produce heads earlier at the highest levels. The plants were pictured before harvesting and are shown in figure 2. At this time all the combinations of salts had caused marked reductions in growth, even at lowest concentration. The data regarding growth, leaf injury, and mortality are presented in table 2. A graph demonstrating growth trend under different treatments at various levels is shown in figure 3. In general, there was a significant decrease in the growth as the salt concentration was increased. It should be noted in figure 2 that the reduction in growth resulting from high concentrations of  $\text{NaCl}$  is less than where  $\text{CaCl}_2$  was the dominant salt. This indicates that the growth was markedly influenced by concentration as

*incipient  
diffusion*UTAH STATE AGRICULTURAL COLLEGE  
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Figure 1. Barley plant growth after two weeks of treatments at isosmotic concentration



Figure 2. Barley plant growth under various treatments at isosmotic concentration before harvesting (three weeks later than figure 1)

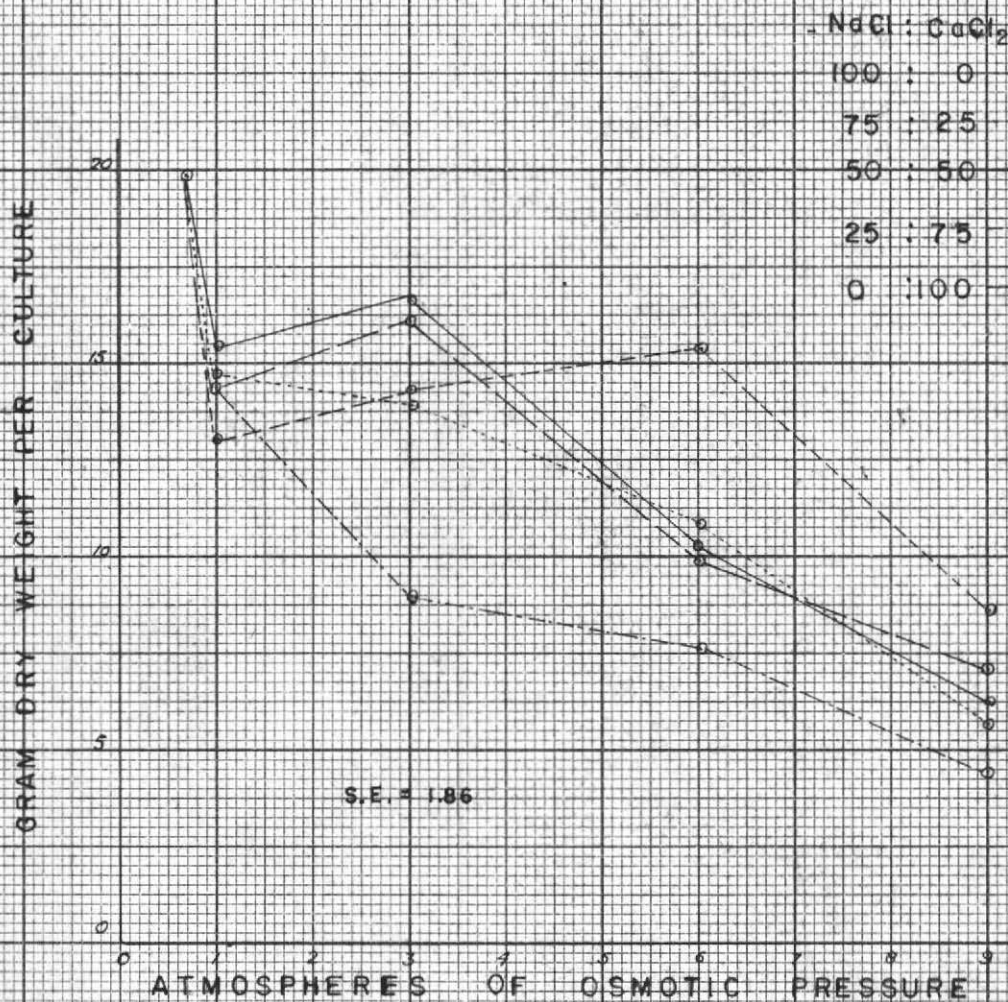
Table 2. The effects of different salt concentrations and proportions of NaCl and CaCl<sub>2</sub> on the yield of barley plants (dry weight in grams)

Treatment identification	Replication	1 (O. P.)			3 (O. P.)			6 (O. P.)			9 (O. P.)		
		Leaves + stem	Roots	Total	Leaves + stem	Roots	Total	Leaves + stem	Roots	Total	Leaves + stem	Roots	Total
NaCl:CaCl <sub>2</sub> 100: 0	I	07.96	1.80	09.76	13.64	2.86	16.50	13.47	3.54	17.01	8.17*	2.12	10.29
	II	12.09	3.06	15.15	07.22	1.72	08.94	15.62	3.94	19.56	7.60*	1.87	9.47
	III	10.92	3.76	14.68	14.76	3.10	17.86	07.66*	2.03	09.69	4.69**†	1.68	6.37
	Av.	10.32	2.87	13.19	11.87	2.56	14.43	12.25	3.17	15.42	6.82	1.89	8.71
75: 25	I	12.51	4.35	16.86	16.63	3.91	20.54	05.15*†	1.95	07.10	9.88*	2.18	12.06
	II	11.92	2.58	14.50	15.28	4.00	19.28	08.95	2.69	11.64	3.03*	0.66	3.69
	III	10.22	2.29	12.51	06.79	1.95	08.74	08.92	2.20	11.12	4.68*	1.20	5.88
	Av.	11.55	3.07	14.62	12.90	3.28	16.18	07.66	2.88	09.96	5.86	1.35	7.21
50: 50	I	16.57	4.38	20.95	15.62	4.32	19.94	11.66*	3.10	14.76	6.47**	1.51	7.98
	II	11.29	3.43	14.72	11.67	3.66	15.33	06.79*	1.86	08.65	6.81**	1.52	8.33
	III	08.55	2.39	10.94	12.21	3.01	15.22	05.93**	1.47	07.40	2.41**†	0.36	2.77
	Av.	12.13	3.40	15.53	13.16	3.66	16.83	08.12	2.14	10.27	5.23	1.13	6.36
25: 75	I	12.90	3.42	16.32	07.09*	2.67	09.76	06.57*	2.03	08.60	4.75**††	1.01	5.76
	II	12.27	4.58	16.85	08.20*	2.55	10.75	05.94**	1.48	07.42	2.20**††	1.06	3.26
	III	07.81	2.48	10.29	05.24*	1.43	06.67	06.13*	1.27	07.40	2.72**††	1.61	4.33
	Av.	10.99	3.49	14.48	06.84	2.22	09.06	06.21	1.59	07.80	3.23	1.23	4.46
0:100	I	13.76	4.52	18.28	14.93*	3.52	18.45	13.10**†	2.42	15.52	3.75**††	1.05	4.80
	II	10.26	2.76	13.02	07.49**	1.63	09.12	04.55**††	1.29	05.84	5.01**††	1.42	6.43
	III	10.61	3.02	13.63	11.62*	3.33	14.95	09.25**†	2.39	11.64	4.55**††	1.33	5.88
	Av.	11.54	3.43	14.97	11.34	2.83	14.17	08.96	2.03	11.00	4.43	1.27	5.70
Control		19.05	6.23	25.28									

\* Intensity of leaf injury.

† Degree of mortality.

# BARLEY



**FIGURE 3.** Yield of barley plants in solution cultures of varied osmotic pressures and salts.

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well as by nature of salts. The percentage of leaf injury and mortality of basal leaves was higher in salts when  $\text{CaCl}_2$  constituted a greater proportion. Tillering of the plants was likewise reduced.

Despite the variability in growth data associated with limited numbers of replications, the treatment effects were relative to leaf injury and mortality, illustrating strikingly the differential influence of the various ions at a given osmotic pressure. It should be noted in this connection, however, that these plants were harvested at incipient flowering and it is possible that further differentiating symptoms in other treatments might have developed during the reproductive phase of growth, and the ultimate fate of the plants would have been worthy of note. However, it appeared that those which suffered severe leaf injury during this period were not going to survive, even though the yield data indicated little or no loss in vegetative vigor.

Leaf injury and scorching were also noticeable on higher levels of  $\text{NaCl}$  salt in later stages of growth. Symptoms, although not very severe, were very much restricted to basal leaves. Plants on  $\text{NaCl}$  series developed a bluish-green color with some waxy coating on the leaves and stems. They were becoming spindly and hardy on higher concentrations of  $\text{NaCl}$ . This quality was decreasing with increasing proportions of  $\text{CaCl}_2$  in the substrate. On the other hand, plants were bright green, soft with broader leaves on all  $\text{CaCl}_2$  treatments, irrespective of concentration. Probably on account of this succulence of tissue some of the plants showed milder attacks of mildew which is considered the characteristic of barley with such morphology under humid conditions.

Inasmuch as specific ion effects were important in relation to the

growth of barley on the saline substrate, the results of this experiment are considered in terms of the accumulation of any of the major inorganic ions in the leaves and roots.

In order to see if there was a relation between the observed injury and accumulation of certain ions within barley plant, analyses were also made on leaves and roots of the barley plants grown in basic Hoagland's nutrient solution to serve as a control for comparison purposes. The distribution of the major ions in both parts of the plant is shown in table 3. Detailed statistical analysis of the yield data and composition is shown in the appendix.

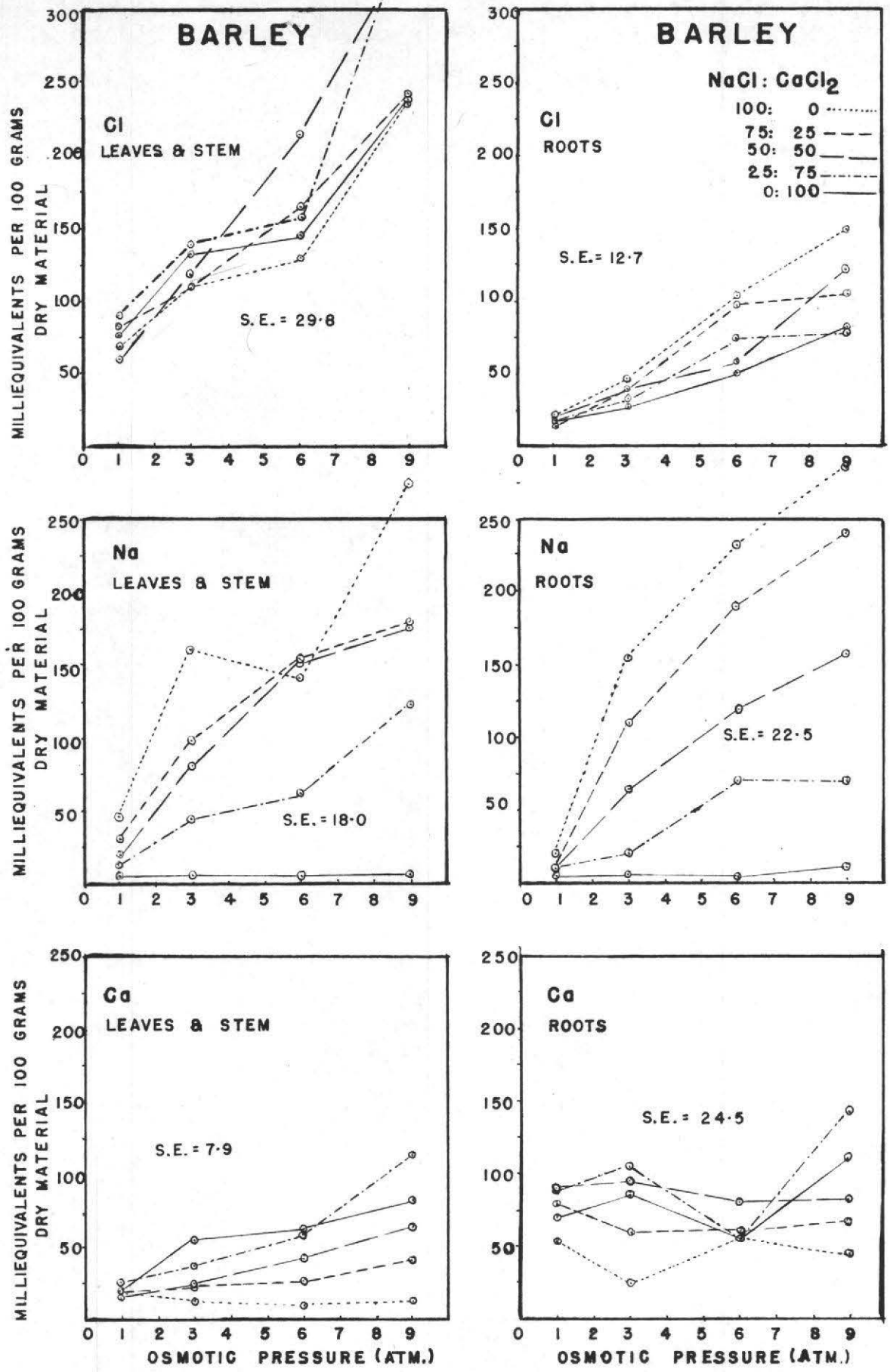
#### Chloride accumulation in barley leaves and roots

The influence of different treatments at various levels on the accumulation of chloride in barley leaves and roots is shown in table 3 and figure 4. Although conditioned by the nature of salt, the chloride concentration in barley leaves generally bore a close relationship to the chloride concentration of the substrate, regardless of whether  $\text{Na}^+$  or  $\text{Ca}^{++}$  was the accompanying cation. This conclusion is in line with the observations of Haas (1950) and Cooper and Gorton (1951). On the basis of data given in table 3, the chloride content of leaves can be calculated with reference to the chloride concentration of the substrate. For each me./liter of chloride in the solution, the leaves on  $\text{CaCl}_2$  treatment accumulated 0.75 me./100 gram dry weight and roots 0.48 me./100 gram dry weight, whereas 0.52 me./100 gram in case of leaves and 0.60 me./100 gram dry weight in case of roots on  $\text{NaCl}$  treatment. The mixed chloride treatments were intermediate according to the proportion of each added salt.

In the roots there were higher concentrations of chloride when

Table 3. The influence of different concentrations and proportions of salts on the composition of barley plants (milliequivalents per 100 grams of dry material)

Treatment identi- fication	O.P.	Na <sup>+</sup>		Ca <sup>++</sup>		K <sup>+</sup>		Mg <sup>++</sup>		Cl <sup>-</sup>		P <sup>----</sup>	
		Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots
NaCl:CaCl <sub>2</sub> 100: 0	1	46.8	21.3	20.6	53.0	145.3	95.4	51.6	27.3	68.6	23.0	23.2	120.3
	3	160.0	154.0	13.7	23.7	78.7	83.4	46.0	32.3	110.3	48.6	29.6	43.7
	6	140.0	232.6	11.6	53.0	65.7	57.4	34.0	19.6	131.0	105.0	30.8	41.2
	9	276.0	288.0	12.7	44.6	74.0	9.6	29.6	12.3	235.0	149.3	30.2	50.3
75: 25	1	27.3	10.2	17.0	79.0	194.7	121.4	46.0	22.3	83.3	15.4	27.9	102.6
	3	98.4	110.0	21.0	59.0	85.3	76.7	40.6	28.6	110.3	42.6	29.3	92.4
	6	158.0	190.6	25.0	60.0	85.0	67.7	28.0	18.6	165.3	99.6	29.7	65.4
	9	178.7	239.0	41.3	66.6	92.0	13.6	29.3	15.0	241.6	104.6	28.0	77.7
50: 50	1	20.0	10.5	17.7	90.3	155.4	130.7	42.0	22.0	60.6	23.0	25.7	111.2
	3	81.0	64.3	24.3	95.3	92.7	132.7	42.6	27.0	118.6	41.6	27.8	99.8
	6	154.0	119.4	42.0	76.3	84.4	69.0	31.0	26.3	214.0	58.3	33.3	97.2
	9	176.6	156.3	64.6	77.0	91.4	31.4	26.3	32.3	378.0	123.6	27.9	82.8
25: 75	1	14.3	11.5	25.0	89.3	195.4	110.0	46.0	29.8	92.6	18.6	27.0	114.0
	3	46.6	20.8	37.7	104.0	108.6	136.0	37.0	25.6	141.6	35.6	30.1	98.1
	6	62.0	70.0	57.0	53.3	90.4	130.7	21.2	27.6	158.3	75.6	29.1	86.8
	9	124.0	69.0	114.6	146.0	110.7	25.4	27.0	31.4	380.0	77.3	26.8	122.5
0:100	1	3.9	6.0	20.7	69.6	139.4	111.7	43.0	27.0	77.6	17.7	30.6	102.7
	3	4.8	4.7	54.6	86.6	158.0	120.0	36.0	26.0	137.6	27.4	27.6	111.8
	6	3.5	4.1	61.5	57.7	111.4	132.0	20.5	23.3	145.6	51.6	27.0	88.7
	9	6.5	11.3	81.3	111.0	104.7	92.6	27.8	40.0	237.3	83.3	32.6	90.0
Control	0.7	3.5	8.0	20.0	110.0	152.00	140.0	41.0	27.0	118.0	57.0	27.4	109.2

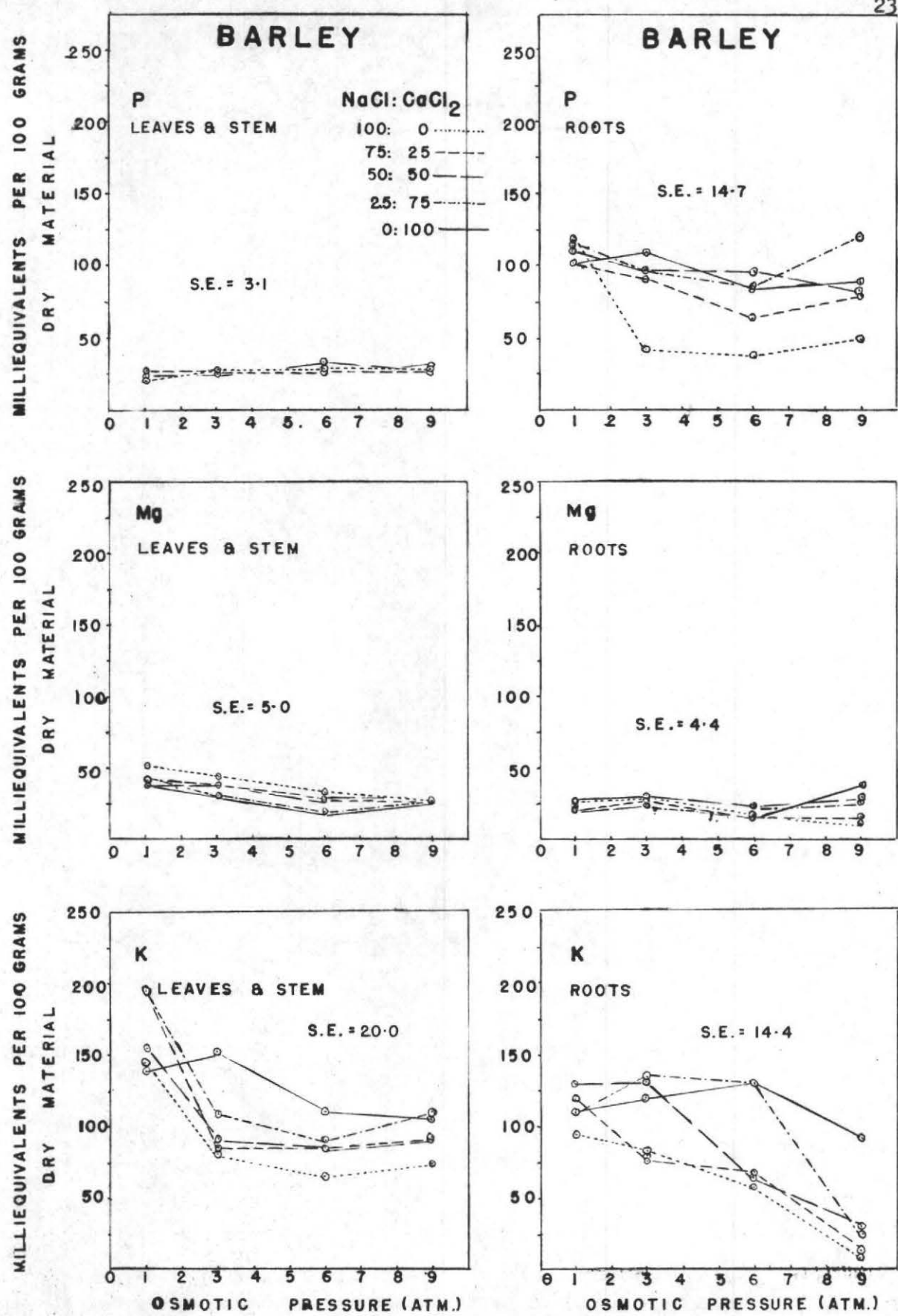


**FIGURE 4.** Effect of treatments on the distribution of ions in various parts of barley plant.

NaCl was greater in proportion as added salt rather than  $\text{CaCl}_2$ . Here, it can be safely concluded that the nature of chloride accumulation in the roots tended to correspond with the trend in absorption of the related cation in a given substrate. These observations are in conformity with the findings of Gauch and Wadleigh (1945) in case of beans and Wadleigh, et al. (1951) in the case of orchard grass. They all indicated that chloride ions are especially toxic to plants in the presence of a high level of calcium. Therefore, severity of symptoms such as leaf burn and eventual death in some cases was closely related to observed levels of chloride accumulation induced by increasing proportions of  $\text{CaCl}_2$  in the substrate.

#### Sodium accumulation in barley leaves and roots

Excess sodium salts caused no lethal effects such as were noted for  $\text{CaCl}_2$ , but leaf injury was observed on higher levels with increasing proportions of NaCl. The concentration of sodium found in the barley in both leaves and roots is shown in table 3 and in figure 5. The data emphasize that barley plants readily accumulate large amounts of sodium both in leaves and roots and that the amount of sodium absorbed and translocated was a function of concentration. Collander (1941) has published extensive data to substantiate his conclusions that some species accumulate more sodium than others and that the proportions of the total cations represented by sodium are characteristic for a given species. The much greater facility with which sodium accumulates in barley is evident. The most striking feature of sodium accumulation in various tissue took place between one and three atmospheres osmotic pressure. Here the sodium accumulation tended to be higher in the upper portion than in the root, whereas further increase of concentration of the substrate reversed the trend, indicating little or no physiological



**FIGURE 5.** Effect of treatments on the distribution of ions in various parts of barley plant.

absorption at highest level. In general, roots tended to accumulate considerably higher percentages of sodium than the tops. There was a definite trend for the sodium content of leaves and roots to decrease with increasing concentrations of calcium in the culture solution. Collander (1941) observed this to be the case in most of the sixteen species of plants he studied. Bower and Wadleigh (1948) found that there was a pronounced accumulation of sodium in the roots of Dallis grass with the increase of sodium salts in the substrate. Similar explanation can be found from Raber's (1923, 1926) electrostatic theory of permeability relating the density of charge on an ion to its effect on protoplasmic permeability in which he postulates that ions with single negative charge make the absorbing membranes less permeable. This theory appears to hold for the effect of the respective anions (in this case chloride) on permeability to sodium in this experiment.

The growth of barley was obviously independent of variations in the content of sodium, as compared to calcium, within the limitations of the values observed. Furthermore, the data do not justify any implication that the sodium ion might be either essential or beneficial to the growth of this species. The data presented in table 3 provide some indication that depressed growth of barley in culture salinized with increasing proportion of sodium chloride might be associated with an abnormally high accumulation of sodium in the tissue, but the fact that isosmotic concentration of calcium chloride and sodium chloride were conducive to practically identical degrees of growth response, regardless of wide disparity in sodium contents, tend to mitigate any idea of a specifically adverse effect of the sodium ion in this cereal. In other words, the effect of increasing proportion of added sodium chloride to the culture solution on barley over and above the intensified

physiological scarcity of water affected by the increased osmotic pressure appears to be nil.

#### Calcium accumulation in barley leaves and roots

The data regarding accumulation of calcium in barley tissues with respect to different treatment are presented in table 3. As can be visualized from data and graph shown in figure 5, the concentration of calcium in both tissues increased directly as the concentration of this element in the culture solution was increased. However, variation in leaves was greater than in roots and there was a tendency for the roots to accumulate calcium as compared to leaves. Despite an increase in the concentration of calcium in the culture solution from 8 me./liter to 305, the concentration of calcium in the entire plant increased only from 30 me./100 gram dry material in the control plant to 88 in the plants grown in the highest concentration of calcium chloride. In other words, the most striking feature of calcium uptake was the moderate degree to which calcium concentration in the barley plant was influenced by wide variations in calcium concentration in the external medium. It is true that the barley plant like other cereals is characterized by a relatively low concentration of calcium in its tissue. Although data emphasize a moderate variation in the accumulation of calcium on the addition of NaCl but it had no effect on calcium concentration in the plant tissue at a given level of calcium supply. Wadleigh, et al. (1951) have reported added calcium chloride to be more inhibitory than NaCl to the growth of orchard grass. Inasmuch as  $\text{Ca}(\text{NO}_3)_2$  produced effects similar to those of  $\text{CaCl}_2$ , they attributed growth depression to excessive calcium accumulation in the plant tissue, whereas this experiment with barley plants suggests a different type of toxicity induced by  $\text{CaCl}_2$ . In this case, the toxic



effect has apparently resulted from increased chloride accumulation and succulence of the tissue in the presence of high concentrations of soluble calcium in the substrate. Consideration of the data on calcium accumulation in leaves as influenced by treatment indicates that excess calcium uptake from substrate high in calcium was not an important factor in the development of injury on these treatments.

Effect of treatments on the accumulation of other ions in barley plants

Data have also been obtained on the accumulation of phosphorous, potassium, and magnesium for both the tissues of plants. Representative data are presented in table 3 and results are graphed in figure 4.

Phosphorous. Earlier work of Eaton (1942) and Gauch and Eaton (1942) have shown that the percentage of phosphorous in plants is very little affected by saline substrate. But the data obtained with respect to the uptake of phosphorous by barley plants have an interesting bearing on this concept. Although increasing proportions of  $\text{CaCl}_2$  at all levels in basal nutrients solution resulted only a little change in phosphorous content of leaves and roots with a tendency to decrease in some cases, but in NaCl series, a very striking decrease took place in the roots. It seems that Gauch and Eaton (1942) based their conclusion on leaf analyses of barley plants only. As indicated by Gauch and Wadleigh (1945), roots were probably not analysed. These data further emphasize the fact that leaf analyses alone may not give complete information regarding the intake and accumulation of ions.

Potassium. Data on potassium accumulation in plants under various treatments are shown in table 3. Culture in nutrient solution is frequently conducive to excessive accumulation of potassium in plants. As a case in point, the control barley was found to contain as much as 152 me. of potassium in leaves alone per 100 grams of dry material.

Although potassium was supplied in equal quantities in all the culture, the added sodium salts definitely limited the accumulation of this ion, whereas potassium concentration was higher from  $\text{CaCl}_2$  treatment. In general, potassium was present in decreasing amounts as the total concentration of the salt was increased. Viets (1946) found that calcium is particularly effective for stimulating potassium absorption. Pierre and Bower (1943) concluded that potassium by plants is usually decreased by the presence of high concentrations of other cations but may be increased. The latter authors pointed out further that other ions affected potassium absorption less than potassium concentration affects the absorption of other ions, particularly calcium and magnesium.

Magnesium. Referring to table 3 again, the increasing proportion of calcium treatments brought about relatively low levels of magnesium in both the tissues of barley while NaCl series practically did not bring out many changes. There was some increase of magnesium in the roots with increasing concentrations of sodium salts. The comparative  $\text{CaCl}_2$  series, however, render unlikely any possibility that such low levels of magnesium approached inadequacy and became limiting to growth.

#### Experiment on Hubam clover

The results in table 4 and figure 6 indicate that, in general, the growth depression of Hubam clover grown on various saline cultures was largely proportional to the osmotic pressure of the respective solutions. No evidence of marked calcium sensitivity was observed at any time. At the highest levels of NaCl, the plants seemed to be extremely sensitive of it and developed chlorotic symptoms which could not be corrected by intensified supplies of ferric tartrate to the substrate. The plants were rather brittle to touch and they were nearly dead at the

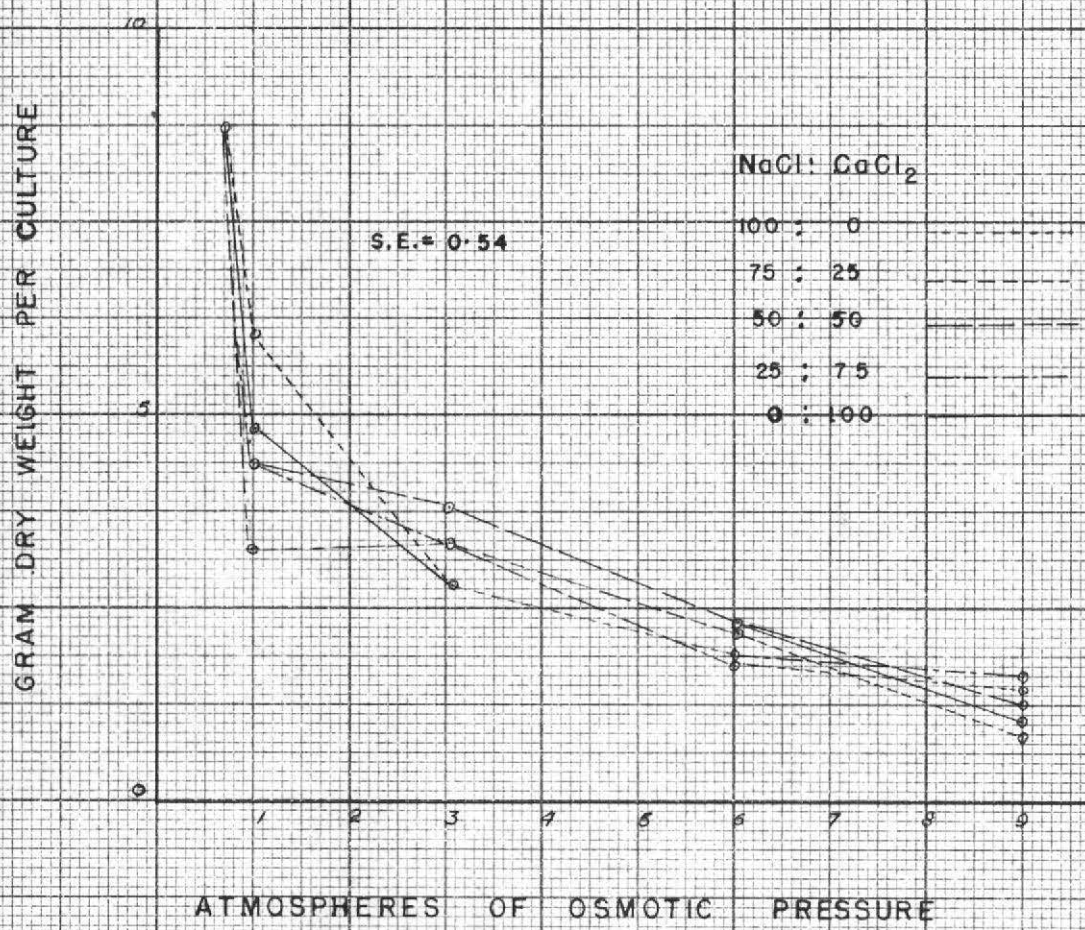
Table 4. The effects of different salt concentrations and proportions of sodium and calcium chloride on the yield of barley plants (dry weight in grams)

Treatment identification	Replication	1 Osmotic Pressure			3 Osmotic Pressure			6 Osmotic Pressure			9 Osmotic Pressure		
		Leaves + stem	Roots	Total	Leaves + stem	Roots	Total	Leaves + stem	Roots	Total	Leaves + stem	Roots	Total
NaCl:CaCl <sub>2</sub> 100: 0	I	1.75	0.70	2.45	2.20	0.95	3.15	1.82	0.84	2.66	0.40*†	0.25	0.65
	II	2.12	0.70	2.82	3.20	1.25	4.45	1.78	0.75	2.53	0.98	0.30	1.28
	III	3.60	1.10	4.70	1.80	0.72	2.52	1.00	0.55	1.55	0.50†	0.26	0.76
	Av.	2.49	0.84	3.32	2.40	0.98	3.38	1.54	0.72	2.26	0.63	0.27	0.90
75: 25	I	4.64	1.89	6.53	3.61	1.47	5.08	1.95	0.75	2.70	0.90	0.40	1.30
	II	2.72	1.05	3.77	1.87	0.72	2.59	1.80	0.67	2.47	0.88	0.37	1.25
	III	2.20	0.85	3.05	2.75	1.12	3.87	1.37	0.55	1.92	0.97	0.40	1.37
	Av.	3.19	1.26	4.45	2.74	1.10	3.84	1.70	0.66	2.36	0.92	0.39	1.31
50: 50	I	3.66	1.50	5.16	2.95	1.10	4.05	1.89	0.80	2.69	1.12	0.51	1.63
	II	4.13	1.35	5.48	1.33	0.52	1.85	1.00	0.45	1.45	0.59†	0.25	0.84
	III	3.13	0.90	4.03	1.78	0.70	2.48	1.96	0.76	2.72	0.90	0.38	1.28
	Av.	3.64	1.25	4.89	2.02	0.77	2.79	1.62	0.67	2.29	0.87	0.38	1.25
25: 75	I	2.59	0.80	3.39	2.70	1.10	3.80	1.31	0.58	1.89	1.33	0.42	1.75
	II	4.48	1.71	6.19	2.35	0.78	3.13	1.30	0.45	1.75	1.15	0.66	1.81
	III	2.90	0.98	3.88	2.10	0.80	2.90	1.20	0.53	1.73	1.00	0.57	1.57
	Av.	3.32	1.16	4.48	2.38	0.89	3.28	1.27	0.52	1.79	1.16	0.55	1.71
0:100	I	4.05	1.45	5.50	1.43	0.50	1.93	1.50	0.75	2.25	1.30	0.47	1.77
	II	6.02	2.25	8.27	2.70	0.95	3.65	1.27	0.40	1.67	1.03	0.35	1.38
	III	3.43	1.25	4.68	2.40	0.72	3.12	1.65	0.52	2.17	1.15	0.45	1.60
	Av.	4.50	1.65	6.15	2.18	0.72	2.90	1.47	0.56	2.03	1.16	0.42	1.58
Control		6.49	2.65	9.14									
	Av.	6.00	2.21	8.21									

\* Intensity of leaf injury.

† Degree of mortality.

### HUBAM CLOVER



**FIGURE 6.** Yield of hubam clover plants in solution cultures of varied osmotic pressures and salts.

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conclusion of the experiment. The appearance of plants just prior to harvesting is shown in figure 7. The most striking feature of this experiment was the similarity of the plants at isosmotic concentration regardless of which combination of salt was added to the base nutrient solution. It should be noted in this connection, however, that these plants were harvested at incipient flowering, and it is possible that some differentiating symptoms in these treatments might have developed during the reproductive phase of growth. The plants were free of any pest or insect attack. The only plants which showed symptoms of malnutrition at the conclusion of the experiment were those subjected to the high concentration of NaCl salt series. The symptoms as already indicated consisted largely of a slight chlorosis near the margins of the leaves.

The results of this experiment are considered in terms of the accumulation of certain ions in both leaves and roots of the plant under various treatments.

#### Chloride accumulation in various tissues of Hubam clover

Data on chloride accumulation in leaves and roots as a result of treatments are presented in table 5. A detailed analysis of the data is given in the appendix. In general there were exponential increases in the concentration of chloride in both the tissues, as the concentration of chloride in the solution was increased, and again for any given level,  $\text{CaCl}_2$  resulted in highest accumulation. The roots generally seemed to exclude this ion with corresponding increase, in leaves. This effect of a predominance of the  $\text{Ca}^{++}$  cation on chloride absorption is typical (Meseawa, 1936). In fact, the relationship is so pronounced that a  $\text{CaCl}_2$  culture solution, similar to the one used herein, proved to be lethal in the barley experiment, and it was extremely deleterious

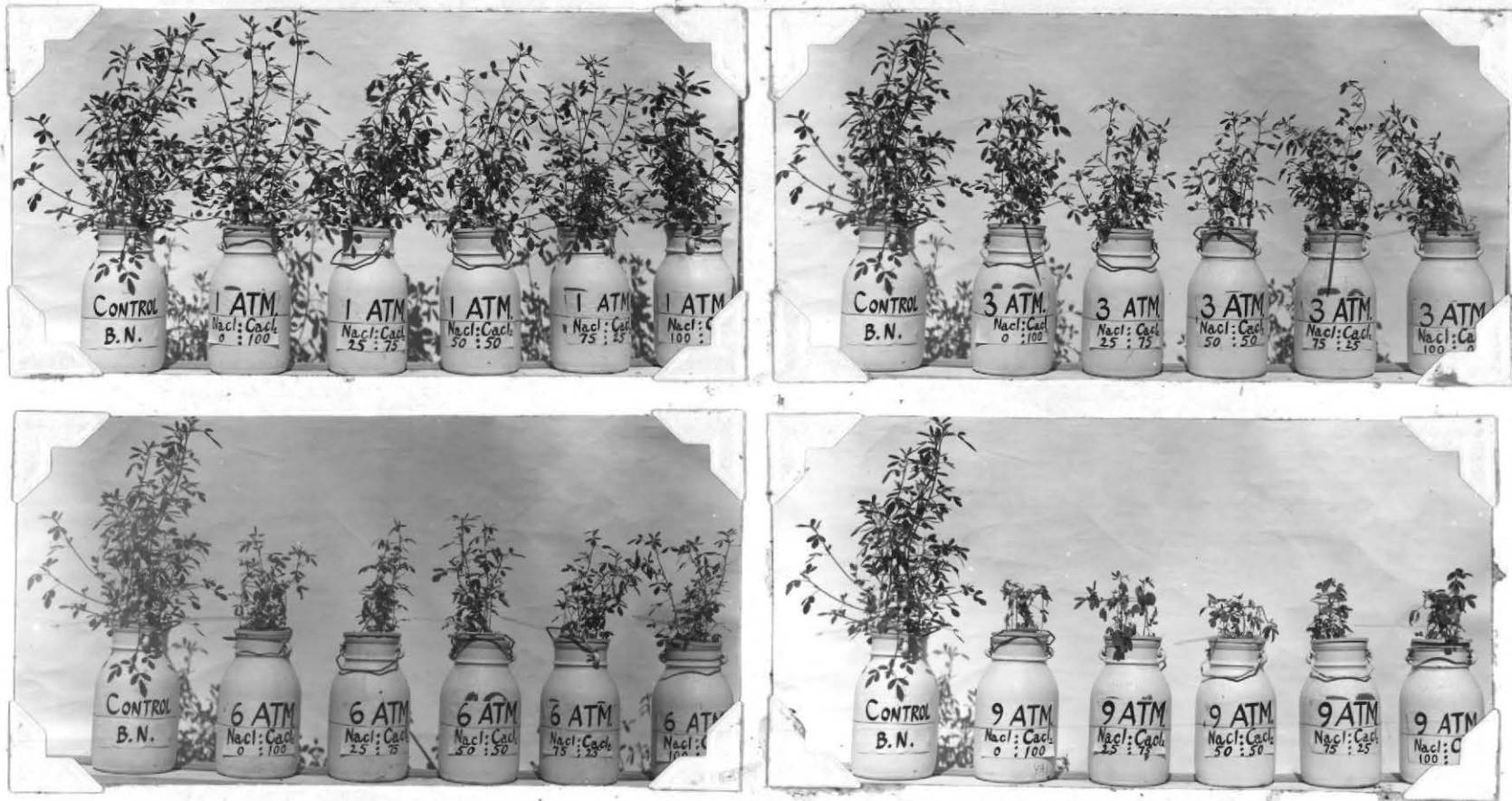


Figure 7. Hubam clover plant growth under various treatments at isosmotic concentration before harvesting

to drupaceous fruit trees (Brown, et al., 1953), as a consequence of excessive chloride toxicity, whereas, an isosmotic solution of added NaCl had comparatively little effect upon the trees. Nevertheless, the evidence at hand from a comparison of various treatment combinations indicates that the highest accumulations of chloride found in both the tissues were not specifically inhibitive to growth.

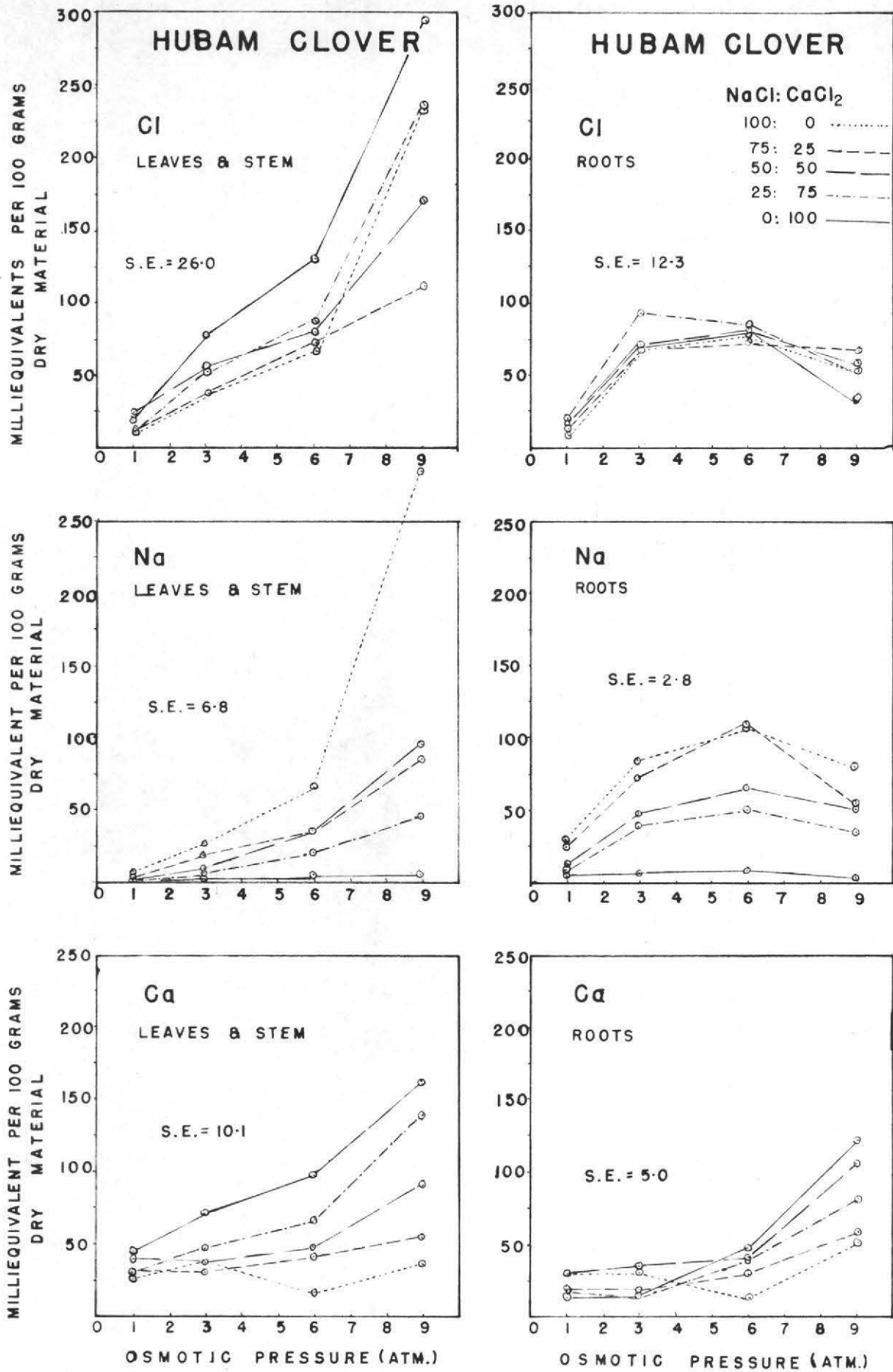
Sodium accumulation in various tissues of Hubam clover

The influence of NaCl salt on the accumulation of sodium in both the tissues is shown in table 5 and figure 8. The addition of NaCl separately and in various mixtures to the basal nutrients solution resulted only in moderate increases in the upper portion of the plant, but a very striking increase in the roots was noted. However, this tendency was almost reversed at the highest concentration. There was a definite trend for sodium content of root tissue to decrease with increasing concentrations of calcium in the culture solution up to the level of 6 atmospheres osmotic pressure; this species exhibited a remarkable mechanism for accumulating sodium in the roots while preventing its accumulation in the leaves at a corresponding level. Collander (1942) has observed that plant species which normally have relatively high concentrations of sodium throughout the plant seem to be the ones that are least sensitive to an increase in sodium concentration in the substrate. Garden beets, sugar beets, atriplex, and other "halophytic" plants are examples of those which may take up large quantities of sodium and are tolerant of relatively high concentrations of sodium in solution. The same author has further indicated that many salt sensitive species of plants are known to take up relatively small amounts of sodium. It is possible that other species of plants which are characterized by a low proportion of sodium in the tops may likewise

Table 5. The influence of different concentrations and proportions of salts on the composition of Hubam clover plants (milliequivalents per 100 grams of dry material)

Treatment identifi- cation	O.P.	Na		Ca		K		Mg		Cl		P	
		Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots	Leaves + stem	Roots
NaCl:CaCl <sub>2</sub> 100: 0	1	6.2	28.7	25.7	28.0	78.7	87.4	30.0	6.2	14.0	8.8	29.4	51.7
	3	25.2	86.3	38.7	30.6	65.0	97.7	34.3	7.6	37.6	68.3	26.2	51.7
	6	65.7	106.0	26.6	24.0	38.7	84.0	27.0	24.0	67.3	76.0	33.5	82.4
	9	288.0	80.0	36.0	50.0	24.0	41.0	32.3	30.0	233.0	53.0	26.4	51.0
75: 25	1	3.5	24.6	32.0	18.6	91.4	102.0	23.0	9.6	15.0	13.0	33.5	59.6
	3	19.4	73.4	30.0	17.6	59.5	98.7	28.3	16.0	38.3	68.0	30.1	61.7
	6	35.4	108.0	42.6	29.0	44.4	116.0	39.0	16.0	72.3	74.0	29.8	82.4
	9	86.0	55.0	53.0	58.0	26.4	67.0	27.0	25.0	110.6	68.5	37.5	62.8
50: 50	1	4.0	14.6	41.3	27.7	78.0	125.0	24.0	9.6	23.0	16.3	23.6	51.5
	3	13.5	47.2	38.0	34.0	54.0	96.0	21.3	39.0	56.0	71.3	30.7	57.8
	6	36.0	65.0	47.3	42.6	42.7	84.7	28.0	27.0	79.0	80.0	31.4	68.0
	9	95.0	50.0	90.3	106.0	49.4	39.0	33.0	33.0	170.0	57.3	33.4	93.2
25: 75	1	2.9	9.8	35.3	21.0	80.6	125.0	19.3	12.3	11.0	19.3	23.1	48.1
	3	8.3	40.5	48.0	15.0	58.0	132.0	13.3	8.5	52.3	95.6	27.3	56.0
	6	20.7	50.6	66.3	38.0	56.0	118.4	7.5	7.0	87.6	86.6	29.2	71.3
	9	47.3	35.5	138.6	82.6	44.4	40.0	8.5	6.0	236.0	53.3	48.7	98.8
0:100	1	1.6	4.1	47.0	14.0	69.7	137.6	20.0	13.0	18.0	16.0	24.0	59.2
	3	1.8	4.0	73.0	15.0	51.7	151.3	7.3	7.0	76.6	71.6	26.5	55.8
	6	2.1	5.2	99.7	48.3	43.3	128.3	5.3	6.6	128.6	83.3	26.6	85.0
	9	3.5	3.5	162.7	122.0	49.4	60.0	4.0	—	294.3	35.0	28.6	103.0
Control	0.7	1.70	3.50	23.00	17.00	100.0	113.0	26.0	47.00	4.0	7.5	24.3	34.20





**FIGURE 8.** Effect of treatments on the distribution of ions in various parts of hubam clover.

have a high proportion of sodium in the roots. Wadleigh and Gauch (1942) observed a difference in the status of the nitrogenous constituents of the root of bean plants as conditioned by high concentration of  $\text{Na}^+$  vs.  $\text{Ca}^{++}$  in the substrate.

It is questionable whether the slightly greater reduction of growth as observed in figure 6 with increasing proportion of NaCl series of plants when compared with  $\text{CaCl}_2$  series is in any way related to the higher concentration of sodium ions, per se, in the dry material of the former series. Because  $\text{CaCl}_2$  resulted in a very similar growth depression at isosmotic levels. Consideration of the data on chloride accumulation in leaves and roots (table 5) indicates that excessive chloride uptake took place in those series where  $\text{CaCl}_2$  constituted the major proportion. Chloride was not an important factor in causing slightly greater reduction in dry material on these treatments.

#### Calcium accumulation in various tissues of Hubam clover

Leguminous plants usually contain appreciably more calcium than do plants of certain other species, viz., the cereals. It was, therefore, conceivable that calcium absorption by Hubam clover would be responsive to wide variation in the concentration of this ion in the growing medium. With an increase in the concentration of calcium in the culture solution from 8 me./liter to 305, the concentration of calcium in the entire plant maintained a corresponding increase from 22 me./100 gram dry material in control plants to 152 in the plant grown in the highest concentration of  $\text{CaCl}_2$  (table 5). However, there were small variations in the accumulations of calcium in roots per level, but the accumulation in leaves maintained a linear increase corresponding to the culture medium. The results in figure 9 confirm that the amount of calcium absorbed and translocated was a function of

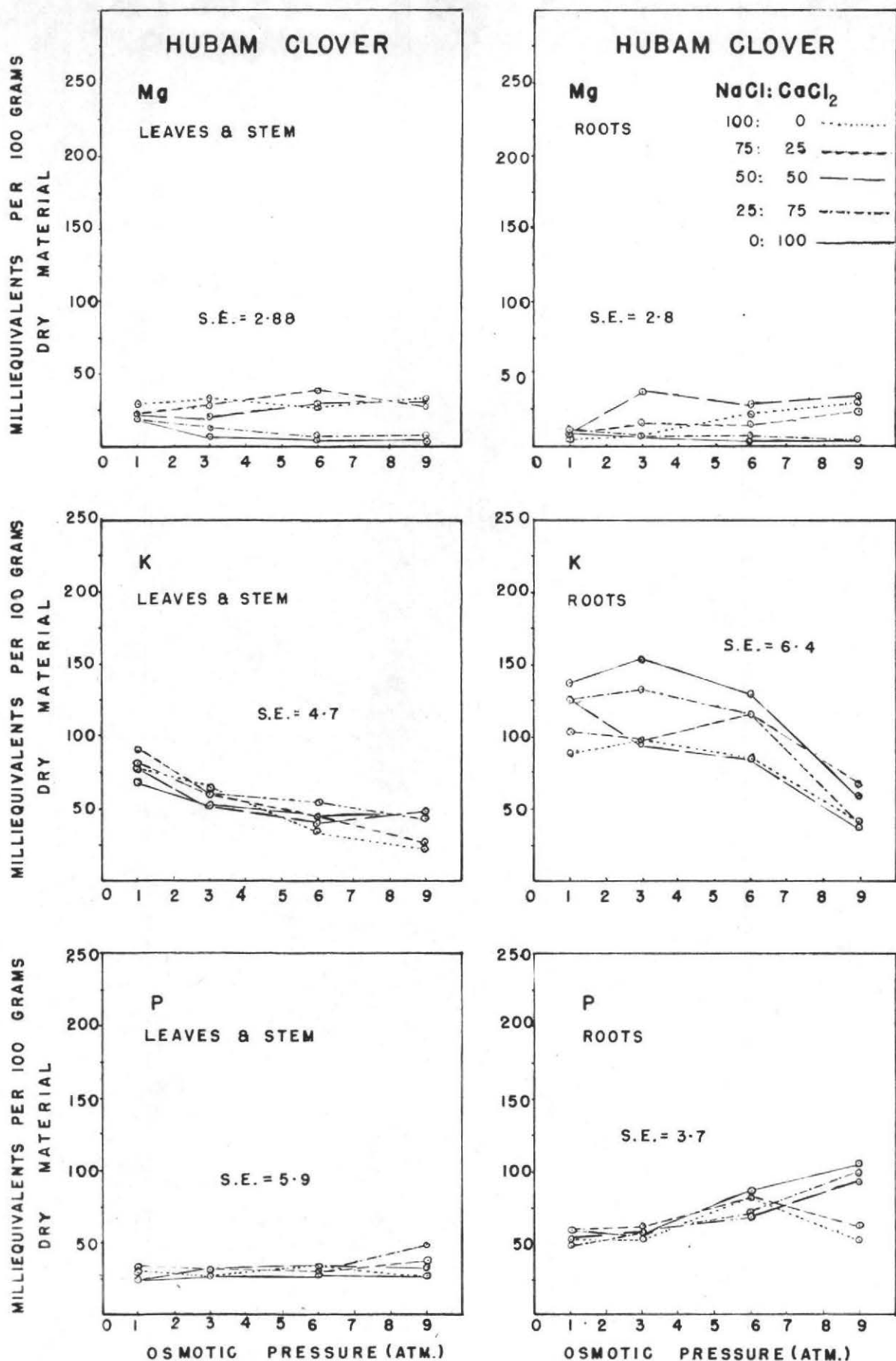


FIGURE. 9. Effect of treatments on the distribution of ions in various parts of hubam clover.

concentration.

Although this legume showed a variable response (table 5) of doubtful significance on the increase of calcium ion, in general the results are in agreement with the concept that osmotic pressure rather than specific ion effects were primarily responsible for decreased yield with added salts.

Effect of concentration of sodium and calcium chlorides on the uptake of other ions by Hubam clover

The presentation and discussion in the preceding section was developed on the basis that the concentration of a given ion in the plant material is a function of the equivalent concentration of that ion in the culture solution. This development was possible in that ions so considered were varied in concentration in the solution. Ions constant in concentration in all treatments cannot, however, be evaluated in this manner. In view of the fact that plant growth in this experiment was more or less closely related to osmotic pressure, it is, therefore, considered to evaluate the accumulation of the ion constant on the basis of the osmotic pressure of these external solutions.

Potassium. There were marked differences in the concentration of potassium in both leaves and roots as the concentration of NaCl and CaCl<sub>2</sub> was varied in the culture solution. Apparently the concentration of potassium in both tissues was conditioned by the kind of salt present in excess. In general, it was noticed that the concentration of potassium in roots was largely governed by the accumulation of the other cations in roots. For example, more sodium was found in the roots of NaCl series than those of CaCl<sub>2</sub> plants, and the content of potassium was just the reverse. As far as CaCl<sub>2</sub> treatment is concerned,

there was an inverse relationship between the concentration of calcium and potassium in the leaves. It is difficult to offer any explanation as to why the highest potassium concentration found in the roots of  $\text{CaCl}_2$  plants is associated with the lowest found in the leaves and vice versa for the  $\text{NaCl}$  series. This may be in accordance with the inter-ionic relationships.

Lidner and Harley (1944), working with the problem of lime-induced chlorosis of pears and apples, noted that the severity of the symptoms were associated with shifts in the K/Ca balance. Similar situations had been frequently observed by Lundegardh (1940) and Hayward and Wadleigh (1949).

Magnesium. The increased concentration of magnesium in the leaves of  $\text{NaCl}$  plants was apparently found to be related to moderate accumulations of sodium concomitant with a pronounced chloride accumulation in the leaves. Bower and Wadleigh (1948) also noticed this effect and reported that magnesium absorption was enhanced by increasing the proportions of absorbed sodium. There were moderate variations in the magnesium contents of root tissue with some variation in calcium supply in the absence of sodium chloride, but when this sodium chloride was added the general level of magnesium in root tissue was lowered. However, the addition of  $\text{NaCl}$  to the substrate at any level was not associated with any definite effect on the magnesium content of the leaves.

Phosphorus. As can be seen from the data in table 5, there was very little effect of either type or amount of added salt on the concentration of phosphorus in different parts of the plant. However, in the  $\text{CaCl}_2$  series the concentration of phosphate in roots showed some

progressive variation with serial increase in the amount of  $\text{CaCl}_2$  in the solution; but it was not considered significant.

## GENERAL DISCUSSION

Perhaps the most outstanding thing demonstrated by this investigation was the marked influence of the nature of salt upon barley response to increasing concentration, whereas the most striking feature in the case of Hubam clover was the close similarity of the plants at isosmotic concentrations regardless of which combination of salt was added to the base nutrient solution. This study, while supporting the concept of osmotic pressure as a factor in plant nutrition, further points out other effects of salt equally important in restricting the growth of certain species. The data on vegetative responses to high osmotic concentrations of sodium chloride and calcium chloride individually and in various combinations indicate that barley is tolerant to sodium salts but sensitive to calcium salts. The effects of these salts on the Hubam clover plants, on the other hand, are of the same order at equal osmotic concentrations.

Reasonably good yields may be expected if the osmotic concentration of the substrate does not exceed 3.0 atm. and poor yields or complete failure in case of barley are probable if the osmotic concentration exceeds 5 or 6 atm., especially in the presence of calcium salts. These conclusions, based on plants grown in solution cultures, correlate well with the observations made by others (Ayers, 1948, and Ayers, et al., 1952).

Hubam clover was purposely included in the experiment to determine whether specific sensitivity to high concentration of calcium salts is responsible for the apparent poor salt tolerance of certain legumes as

judged by performance in artificially salinized plots conducted by Ayers (1948). When dry weights of forage produced were plotted against osmotic pressure of the solutions, the locus of points reasonably approximated a straight line for all the combinations of salts.

Excessive leaf injury produced by higher accumulation of chloride, when calcium was the complementary cation, was probably associated with fineness and succulence of the barley tissue produced by the calcium salts. Because similar concentrations of chloride on the sodium-treated plants did not produce the same effects. Plant morphology was different in the presence of excessive sodium salts. They were comparatively hardy with usual waxy coating on them. The excessive depression in growth of barley plants on calcium treatment was associated with reduction of leaf functioning area due to excessive leaf injury.

The data pertaining to the mineral composition of the plants in both the species show a definite increase in ion uptake with increased concentration. This general trend indicated by plant analysis is contrary to the findings of Olsen (1950) where he concluded that the rate of ion absorption is independent of the concentration. Further examination of the data indicates greater variations in uptake of different constituents by these two species. For instance, there was a marked difference in calcium absorption and its further translocation between both the species. In other words, with an increase in the concentration of this ion, the amount taken up by barley was found substantially less than that by Hubam clover. Again, a decrease in total calcium uptake with decrease in calcium concentration was noted most markedly in case of barley and least with Hubam clover. Although this effect slowly vanished at higher concentration. A similar trend was noted for both



the species with respect to sodium absorption at different concentrations. Generally, greater proportion of sodium at higher concentration depressed total calcium uptake, but the effect was very much restricted to low accumulation in the roots as there was no proportional decrease of calcium in the tops. At low concentrations of sodium there was either little effect or slight stimulation of calcium uptake.

This differential uptake of monovalent and divalent cations by plant roots with different cation exchange capacities supports Mattson's theory (1948), which proposed that at lower concentrations the roots with high density of charge should take up relatively more of divalent cations than a root with a low density of charge. Elgabaly and Wiklander (1949) have used valence effect to predict differential monovalent and divalent cation uptake by roots of the same plant from different clays and by the roots of different plants from the same clay. Pea roots (71 me./100 gm.) and barley roots (22.7 me./100 gm.) were placed in Na-Ca bentonite systems for 10 hours. Pea roots absorbed two to three times as much calcium as did barley roots, while barley roots absorbed four to five times as much sodium as did pea roots. Although cation uptake by plants from nutrient solution omits this important competition of soil colloid with plant root colloid for adsorbed cations, the property of cation exchange capacity of roots plays a similar role in absorption and seems to obey the same laws in solution. Enhanced calcium absorption by Hubam clover over barley partially explains that with increasing cation exchange capacity there is an increase in the bonding energy of calcium (Mehlich and Drake, 1955) while greater absorption of sodium over calcium with low cation exchange capacity is found true for barley. Adopting Mattson's idea of differential cationic uptake in explaining the relative uptake of mono- and divalent cations

by plants, it can be seen that the results of the present investigation coincide with the findings of others, at least under lower levels. At higher levels, this valence effect seems to be destroyed by greatly increasing the outside cation concentration. Broyer and Hoagland (1943) found that roots subjected to high salts lost their ability to exclude ions and the movement of salts to the tops was in same concentration as occurred in the nutrient media. Drake (1951), from his experiments on different crops, concluded that with an increase of free electrolytes the inequalities of the Donnan distribution of ions in plants is evened out. The results obtained in this investigation are quite in line with Drake's conclusion.

The effect of a predominance of the calcium cation on chloride absorption is typical and is not easily explained on the basis of differences in root cation capacity or of the Donnan Principle. This effect probably is related to the protoplasmic permeability and metabolic-physiological process in the plant itself.

### SUMMARY AND CONCLUSIONS

1. The investigation was undertaken to attain, by means of solution culture, a better understanding of the significance of specific salt toxicity of higher concentrations of sodium chloride and calcium chloride in relation to the growth and mineral composition of barley and Hubam clover.

2. Sodium chloride and calcium chloride were added in the following ratios: 100:0, 75:25, 50:50, 25:75, 0:100, to the base nutrient solution in quantities sufficient to raise the osmotic pressure by increments of 1, 3, 6, and 9 atmospheres.

3. Calcium chloride was more toxic to barley than isosmotic levels of sodium chloride, while no such toxicity was exhibited in case of Hubam clover.

4. The addition of different combinations of salts to the base nutrient solution affected not only the concentration of the ions of the added salt in the plant but in some cases the uptake of base nutrient ions as follows:

(a) Sodium. Sodium contents of barley leaves was higher than the roots at lower concentrations but the roots accumulated more sodium at high concentrations, whereas sodium contents in different parts of Hubam clover were characteristically low and increased steadily with increasing concentrations of sodium in the substrate.

(b) Calcium. The increasing concentration of calcium chloride in the combination resulted in the exclusion of calcium from the leaves of barley at higher osmotic levels with an accompanying increase in the

roots. Strikingly, the effect of the addition of sodium chloride progressively decreased the concentration of calcium in the leaves as the amount of this salt was increased in the combination. Hubam clover showed an increase in the concentration of calcium in both the parts of the plant, but the increase was by no means proportional to the amount added.

(c) Chloride. Both the species accumulated considerable quantities of chloride. In the leaves of barley there were higher concentrations of chloride when calcium chloride was greater in proportion than sodium chloride, indicating that calcium stimulated chloride absorption. Severity of symptoms such as leaf burn and eventual death in some cases was closely related to observed levels of chloride accumulation in the tissue induced by increasing proportions of calcium chloride in the substrate.

(d) Potassium. Potassium uptake in both the species was conditioned by the kind of salt present in excess. In general, potassium was present in decreasing amount as the total concentration of the salt was increased. This was most pronounced where sodium salts dominated the substrate.

(e) Magnesium. Increasing proportion of calcium chloride treatments brought relatively low levels of magnesium in both the roots and tops of barley, while addition of sodium chloride practically did not bring out many changes. The increased concentration of magnesium in the leaves of Hubam clover under higher concentration of sodium chloride was associated with moderate accumulation of sodium. Slight variation of this ion in the root contents of both the species was non-significant.

(f) Phosphorus. The addition of salts in any combination to the

base nutrient solution regardless of the type or concentrations employed had very little effect on phosphate content of Hubam clover. Although increasing concentration of calcium chloride to the substrate resulted in little change in barley, with the increasing concentration of sodium chloride a very striking increase took place in the roots.

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**APPENDIX**

The data of the text were statistically analyzed and presented in the following tables. The general trends of significance are noted in tables 1, 3, 4, 12, and 13. Summary tables were also prepared for dry weight yields, and each of the chemical constituents determined such as given in table 2. In these tables the standard error for comparison of individual values is given, as are the standard errors for mean differences of treatments and mean differences for the different osmotic concentrations.

The standard error values shown on the figures in the body of the text were taken from the values reported on these tables.

Table 1. Analysis of variance on total dry weight of barley and Hubam clover

Source of variation	M E A N   S Q U A R E S		
	D. F.	Barley Dry weight	Hubam clover Dry weight
Replications	2	69.77**	0.99
Treatments	4	28.12*	0.83
L	1	43.14*	1.82
Q	1	9.70	0.02
C	1	25.48	1.34
Q	1	34.19	0.10
Osmotic pressure	3	209.22**	30.89**
L	1	566.31**	91.19**
Q	1	59.18*	1.48
C	1	2.17	0.00
Treat. x O. P.	12	11.09	1.88*
LL	1	23.92	1.89
LQ	1	13.23	7.48**
LC	1	0.07	0.45
QL	1	18.09	0.06
QQ	1	8.03	0.00
QC	1	17.50	0.01
CL	1	2.43	1.04
CQ	1	6.49	0.08
CC	1	27.50	0.20
QL	1	0.16	0.16
QQ	1	12.91	0.30
QC	1	2.74	1.41
Error	38	10.38	0.87
Total	59		
L. S. D.		5.32	1.54

Table 2. The effect of different proportions of salt and osmotic concentrations on the average dry weight of the entire plant and their standard errors

(a) Barley

S. E. = 1.8601

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	13.19	14.43	15.42	8.71	12.94
	2	14.62	6.19	9.95	7.21	11.99
	3	15.54	16.83	10.27	6.36	12.24
	4	14.49	9.06	7.81	4.45	8.95
	5	14.98	14.17	11.00	5.70	11.46
$\bar{x}$		14.56	14.14	10.89	6.49	11.52

S. E. = 0.9300

S. E. = 0.8306

(b) Hubam clover

S. E. = 0.5385

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	3.32	3.37	2.25	0.90	2.46
	2	4.45	3.85	2.36	1.31	2.99
	3	4.89	2.79	2.29	1.25	2.80
	4	4.49	3.28	1.79	1.71	2.82
	5	6.15	2.90	2.03	1.58	3.17
$\bar{x}$		4.66	3.24	2.14	1.35	2.85

S. E. = 0.2692

S. E. = 0.2408

Table 3. Analysis of variance for yield and chemical composition of barley tops (leaves and stems), showing mean squares for all sources of variation

Source of variations	D. F.	Dry weight	Sodium	Calcium	Potassium	Magnesium	Chloride	Phosphorus
Replication	2	45.37**	14.93	4.48	46.17*	0.67	62.79	0.75
Treatments	4	21.48*	401.59**	41.53**	28.50	1.76	85.94*	0.03
L	1	32.41*	1522.33**	151.25**	90.48*	6.69**	58.38	0.036
Q	1	8.45	44.54*	3.63	1.64	0.00	213.30**	0.023
C	1	20.30	22.82	7.57	2.16	0.24	63.65	0.027
Q	1	25.12	16.65	3.69	19.71	0.11	8.44	0.016
Osmotic pressure	3	127.43**	438.50**	50.01**	193.90**	14.41**	131.58**	0.36
L	1	335.80**	1292.18**	141.53**	402.75**	39.11*	3603.25**	0.48
Q	1	44.72*	1.82	6.76	176.13**	0.75	268.39**	0.27
C	1	1.79	21.51	1.74	2.84	3.36*	75.90	0.02
Treat. x O. P.	12	6.79	45.59**	8.36**	12.65	0.21	45.16	0.18
LL	1	12.43	333.77**	61.64**	5.34		12.13	
LQ	1	6.93	1.11	0.10	33.07		2.91	
LC	1	0.84	33.07	1.70	18.09		14.14	
QL	1	10.95	37.89	13.75*	5.47		239.47**	
QQ	1	6.90	11.43	5.59	37.15		29.92	
QC	1	9.85	50.34*	0.31	13.53		3.59	
CL	1	1.58	15.63	6.47	0.00		80.67	
CQ	1	2.89	24.22	5.74	5.46		58.96	
CC	1	19.86	32.34	0.12	0.00		32.37	
QL	1	0.03	5.38	2.10	11.83		60.29	
QQ	1	7.94	1.60	2.08	19.41		2.16	
QC	1	1.26	1.10	0.65	2.84		5.19	
Error	38	6.76	9.74	1.87	12.08	0.74	26.68	0.29
Total	59							
L. S. D.		4.30	5.16	2.25	5.74	1.42	8.53	0.89

Table 4. Analysis of variance for yield and chemical composition of barley roots, showing mean squares for all sources of variation

Source of variations	D. F.	Dry weight	Sodium	Calcium	Potassium	Magnesium	Chloride	Phosphorus
Replication	2	2.62*	13.22	16.91	21.22*	0.74	30.80**	9.12
Treatments	4	0.46	507.70**	50.56*	56.39**	0.98	23.45**	32.36**
L	1	0.83	2014.25**	94.44*	221.95**	0.39	90.30	96.62**
Q	1	0.05	5.53	90.70*	0.00	1.22	1.29	32.00*
C	1	0.29	3.87	15.07	0.92	2.14	0.97	0.62
Q	1	0.69	7.15	2.03	2.69	0.18	1.23	0.21
Osmotic pressure	3	10.32**	484.67**	12.78	200.30**	1.56	233.19**	31.80**
L	1	29.90**	1368.66**	0.04	492.29**	3.14*	689.78	60.49**
Q	1	1.05	77.25*	22.23	104.28**	0.45	3.70	33.33*
C	1	0.01	8.11	16.09	4.34	1.09	6.07	1.58
Treat. x O. P.	12	0.67	63.65*	11.79	15.59	1.16	7.44	8.63
LL	1	1.86	619.64**	20.91	45.16*		50.63**	26.29*
LQ	1	1.01	64.15*	0.05	15.99		0.14	18.75
LC	1	0.29	0.29	54.08	1.02		1.24	76.16**
QL	1	1.30	0.31	0.01	46.48**		0.91	8.65
QQ	1	0.01	0.75	1.69	1.52		2.50	6.03
QC	1	1.22	9.43	9.04	2.72		0.36	5.89
CL	1	0.09	9.48	17.02	1.29		0.00	0.98
CQ	1	0.82	18.12	3.63	40.37*		0.08	17.19
CC	1	0.62	0.13	0.83	0.76		0.00	3.24
QL	1	0.06	15.55	10.36	0.38		3.51	4.32
QQ	1	0.66	16.76	19.65	6.24		23.80*	4.09
QC	1	0.10	9.18	0.00	25.21		14.62	0.51
Error	38	0.53	15.18	18.06	6.24	0.57	4.87	6.48
Total	59							
L. S. D.		1.20	6.43	7.03	4.13	1.25	3.64	4.21



Table 5. The effect of different proportions of salt and osmotic concentration on the average dry weight of various parts of barley plant and their standard errors

(a) Leaves and stem

S. E. = 1.5000

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	10.32	11.87	12.25	6.82	10.32
	2	11.55	12.90	7.67	5.86	9.50
	3	12.14	13.16	8.13	5.23	9.66
	4	10.99	6.84	6.21	3.22	6.82
	5	11.54	11.35	8.97	4.44	9.07
$\bar{x}$		11.31	11.23	8.65	5.11	9.07

S. E. = 0.748

S. E. = 0.6708

(b) Roots

S. E. = 0.4123

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.87	2.56	3.17	1.89	2.62
	2	3.07	3.29	2.28	1.35	2.50
	3	3.40	3.66	2.14	1.13	2.58
	4	3.49	2.22	1.59	1.23	2.13
	5	3.43	2.83	2.03	1.27	2.39
$\bar{x}$		3.25	2.91	2.24	1.37	2.44

S. E. = 0.209

S. E. = 0.189

Table 6. The effect of different proportions of salts and osmotic concentration on the uptake of sodium by various parts of barley plant and their standard errors

(a) Leaves and stem

S. E. = 1.8027

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	4.68	16.00	14.07	27.60	15.59
	2	2.73	9.83	15.80	17.87	11.56
	3	2.00	8.10	15.40	17.67	10.79
	4	1.43	4.68	6.20	12.40	6.18
	5	0.39	0.48	0.35	0.65	0.47
	$\bar{x}$	2.25	7.82	10.36	15.24	8.92

S. E. = 0.806

S. E. = 0.90

(b) Roots

S. E. = 2.2494

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.13	15.40	23.27	28.80	17.40
	2	1.03	11.00	19.07	15.93	11.76
	3	1.05	6.43	11.93	15.63	8.76
	4	1.15	2.08	7.00	6.90	4.28
	5	0.60	0.47	0.41	11.33	0.65
	$\bar{x}$	1.19	7.08	12.33	13.68	8.57

S. E. = 1.004

S. E. = 1.118

Table 7. The effect of different proportions of salt and osmotic concentration on the uptake of calcium by various parts of barley plant and their standard errors

(a) Leaves and stem

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.07	1.37	1.17	1.27	1.47
	2	1.70	2.10	2.50	4.13	2.61
	3	1.77	2.43	4.20	6.47	3.72
	4	2.50	3.77	5.70	11.47	5.86
	5	2.07	5.47	6.15	8.13	5.45
	$\bar{x}$	2.02	3.03	3.94	6.29	3.82

S. E. = 0.7874

S. E. = 0.352

S. E. = 0.395

(b) Roots

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	5.31	2.37	5.30	4.47	4.36
	2	7.97	5.90	6.00	6.67	6.63
	3	9.03	9.53	7.63	7.70	8.47
	4	8.93	10.40	5.33	14.63	9.82
	5	6.97	8.67	5.77	74.00	7.20
	$\bar{x}$	7.64	7.37	6.01	8.17	7.30

S. E. = 2.453

S. E. = 1.095

S. E. = 1.224

Table 8. The effect of different proportions of salt and osmotic concentration on the uptake of potassium by various parts of barley plant and their standard errors

(a) Leaves and stem

S. E. = 2.0074

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	14.53	7.87	6.56	7.40	9.09
	2	19.47	8.53	8.50	9.20	11.42
	3	15.53	9.27	8.43	9.13	10.59
	4	19.53	10.87	9.03	11.07	12.62
	5	13.93	15.80	11.13	10.47	12.83
$\bar{x}$		16.60	10.47	8.73	9.45	11.31

S. E. = 0.894

S. E. = 1.00

(b) Roots

S. E. = 1.4387

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	9.53	8.33	5.73	0.97	6.14
	2	12.13	7.67	6.77	1.37	6.98
	3	13.07	13.27	6.90	3.13	9.09
	4	11.00	13.60	13.07	2.53	10.05
	5	11.17	12.00	13.20	9.27	11.41
$\bar{x}$		11.38	10.97	9.13	3.45	8.73

S. E. = 0.645

S. E. = 0.721

Table 9. The effect of different proportions of salt and osmotic concentration on the uptake of magnesium by various parts of barley plant and their standard errors

(a) Leaves and stem

S. E. = 0.5000

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	5.16	4.60	3.37	2.97	4.02
	2	4.58	4.07	2.77	2.93	3.59
	3	4.20	4.25	3.13	2.63	3.55
	4	4.62	3.69	2.14	2.73	3.29
	5	4.33	3.68	2.09	1.85	2.99
	$\bar{x}$	4.58	4.06	2.70	2.62	3.49

S. E. = 0.223

S. E. = 0.248

(b) Roots

S. E. = 0.4358

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.73	3.23	1.93	1.23	2.28
	2	2.23	2.87	1.87	1.50	2.12
	3	2.23	2.67	2.57	3.23	2.67
	4	2.99	2.53	2.43	3.11	2.77
	5	2.70	2.63	2.30	1.33	2.24
	$\bar{x}$	2.57	2.79	2.22	2.08	2.42

S. E. = 0.195

S. E. = 0.2179

Table 10. The effect of different proportions of salt and osmotic concentration on the uptake of chloride by various parts of barley plants and their standard errors

(a) Leaves and stem

S. E. = 2.9816

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	6.87	11.03	13.10	23.50	13.62
	2	8.33	11.03	16.53	24.17	15.02
	3	6.07	11.87	21.40	37.87	19.30
	4	9.27	14.17	15.83	38.03	19.32
	5	7.77	13.77	14.57	23.73	14.96
	$\bar{x}$	7.66	12.37	16.28	29.46	16.44

S. E. = 1.334

S. E. = 1.4899

(b) Roots

S. E. = 1.2727

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.30	4.87	10.50	14.93	8.15
	2	1.53	4.27	9.97	10.47	6.56
	3	2.30	4.17	5.83	12.37	6.17
	4	1.87	3.57	7.57	7.73	5.18
	5	1.77	2.73	5.17	8.33	4.50
	$\bar{x}$	1.95	3.92	7.81	10.77	6.11

S. E. = 0.570

S. E. = 0.637

Table 11. Effect of different proportions of salt and osmotic concentration on the uptake of phosphorus by various parts of barley plant and their standard errors

(a) Leaves and stem

S. E. = 0.3114

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.32	2.96	3.08	3.01	2.84
	2	2.79	2.83	2.97	2.80	2.85
	3	2.57	2.78	3.33	2.78	2.87
	4	2.70	3.01	2.91	2.69	2.82
	5	3.06	2.75	2.70	3.26	2.94
	$\bar{x}$	2.69	2.86	2.99	2.91	2.86

S. E. = 0.142

S. E. = 0.155

(b) Roots

S. E. = 1.4696

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	12.03	4.37	4.12	5.03	6.38
	2	10.27	9.24	6.53	7.77	8.45
	3	11.12	9.98	9.72	8.28	9.77
	4	11.40	9.81	8.68	12.25	10.54
	5	10.27	11.19	8.87	9.00	9.83
	$\bar{x}$	11.02	8.92	7.58	8.49	8.99

S. E. = 0.657

S. E. = 0.735

Table 12. Analysis of variance for yield and chemical composition of Hubam clover tops (leaves and stems), showing mean squares for all sources of variation

Source of variations	D. F.	Dry weight	Sodium	Calcium	Potassium	Magnesium	Chloride	Phosphorus
Replication	2	0.43	2.17	17.04**	0.68	0.91*	44.15	0.23
Treatments	4	0.50	150.21**	83.25**	1.01	12.29**	78.57**	0.78
L	1	1.27	500.41**	323.87**	2.09	44.38**	174.24**	0.36
Q	1	0.01	38.39**	8.75	1.33	1.43*	113.69*	0.36
C	1	0.73	45.57**	0.38	0.23	1.99*	13.80	0.01
Q	1	0.00	16.47**	0.01	0.49	1.37*	12.55	1.10
Osmotic pressure	3	17.41**	308.25**	99.44**	54.13**	0.21	1049.69**	1.92
L	1	51.28**	764.96**	245.62**	150.45**	0.38	2813.98**	5.28*
Q	1	0.96	144.56**	31.54**	11.75**	0.24	276.49**	0.44
C	1	0.00	15.21**	12.18	0.19	0.03	58.60	0.04
Treat. x O. P.	12	0.52	75.18**	15.21**	2.22**	1.20**	32.02	0.84
LL	1	1.03	528.02**	149.98**	11.86**	4.36**	90.79*	1.44
LQ	1	3.50**	131.02**	10.31	3.38*	0.97	0.36	0.08
LC	1	0.19	20.20**	0.16	0.77	0.00	1.68	0.88
QL	1	0.04	64.82**	0.33	0.43	2.28**	147.34*	1.71
QQ	1	0.00	34.54**	3.73	0.22	0.10	36.03	0.33
QC	1	0.00	5.87*	3.22	0.64	0.30	5.57	0.71
CL	1	0.34	62.19**	2.76	1.72	0.84	51.21	2.11
CQ	1	0.02	22.86**	1.94	1.24	1.19*	35.67	0.02
CC	1	0.11	1.70	8.41	0.00	1.65*	10.17	0.00
QL	1	0.13	22.64**	1.19	3.62*	0.66	1.08	0.61
QQ	1	0.22	8.20*	0.45	1.47	1.42*	2.99	2.14
QC	1	0.69	0.40	0.09	1.37	0.02	1.61	0.11
Error	38	0.45	1.39	3.08	0.67	0.25	20.31	1.05
Total	59							
L. S. D.		1.11	1.95	2.90	1.36	0.84	2.36	1.69



Table 13. Analysis of variance for yield and chemical composition of Hubam clover roots, showing mean squares for all sources of variation

Source of variations	D. F.	Dry weight	Sodium	Calcium	Potassium	Magnesium	Chloride	Phosphorus
Replication	2	0.12	0.34	1.77	4.36*	0.28	2.43	0.68
Treatments	4	0.05	94.06**	13.80**	31.82**	7.45**	15.04**	4.20**
L	1	0.05	363.00**	29.86**	102.86**	10.09**	31.42*	14.46**
Q	1	0.01	7.47**	2.73	3.57	7.97**	9.52	0.01
C	1	0.10	0.99*	0.21	8.43*	0.40	18.45	2.33*
Q	1	0.03	4.79**	22.41**	12.43**	11.36**	0.79	0.00
Osmotic pressure	3	1.92**	66.25**	131.79**	150.30**	1.98**	98.59**	29.90**
L	1	5.70**	78.39**	297.76**	320.33**	5.57**	57.42**	79.70**
Q	1	0.05	116.62**	94.05**	118.16**	0.18	191.35**	0.03
C	1	0.00	3.75**	3.58*	12.40**	0.20	47.00**	9.97**
Treat. x O. P.	12	0.11	6.35**	7.64**	6.00**	2.13**	7.55	4.85**
LL	1		22.22**	70.11**	23.02**	12.32**	0.00	23.34**
LQ	1		37.30**	5.37*	7.65*	0.00	36.30**	15.54**
LC	1		0.70	1.45	0.35	0.06	34.56**	3.77**
QL	1		1.34*	0.00	0.75	0.03	0.96	0.76
QQ	1		2.99**	0.01	5.63*	1.42*	0.40	1.50
QC	1		0.73	0.01	4.11	3.21**	1.66	6.24**
CL	1		1.35*	3.14*	9.88**	0.08	1.44	2.44*
CQ	1		0.61	0.07	1.78	0.00	4.70	0.48
CC	1		2.90**	0.04	1.04	0.44	0.03	0.83
QL	1		0.09	5.95*	7.82*	2.26**	2.74	1.21
QC	1		5.22**	1.84	9.18**	2.00**	0.08	1.57
QC	1		0.75	3.16*	0.79	0.69	8.98	0.52
Error	38	0.07	0.24	0.75	1.22	0.24	4.56	0.43
Total	59							
L. S. D.		0.44	0.81	1.43	1.83	0.81	3.53	1.09

Table 13. Analysis of variance for yield and chemical composition of Hubam clover roots, showing mean squares for all sources of variation

Source of variations	D. F.	Dry weight	Sodium	Calcium	Potassium	Magnesium	Chloride	Phosphorus
Replication	2	0.12	0.34	1.77	4.36*	0.28	2.43	0.68
Treatments	4	0.05	94.06**	13.80**	31.82**	7.45**	15.04**	4.20**
L	1	0.05	363.00**	29.86**	102.86**	10.09**	31.42*	14.46**
Q	1	0.01	7.47**	2.73	3.57	7.97**	9.52	0.01
C	1	0.10	0.99*	0.21	8.43*	0.40	18.45	2.33*
Q	1	0.03	4.79**	22.41**	12.43**	11.36**	0.79	0.00
Osmotic pressure	3	1.92**	66.25**	131.79**	150.30**	1.98**	98.59**	29.90**
L	1	5.70**	78.39**	297.76**	320.33**	5.57**	57.42**	79.70**
Q	1	0.05	116.62**	94.05**	118.16**	0.18	191.35**	0.03
C	1	0.00	3.75**	3.58*	12.40**	0.20	47.00**	9.97**
Treat. x O. P.	12	0.11	6.35**	7.64**	6.00**	2.13**	7.55	4.85**
LL	1		22.22**	70.11**	23.02**	12.32**	0.00	23.34**
LQ	1		37.30**	5.37*	7.65*	0.00	36.30**	15.54**
LC	1		0.70	1.45	0.35	0.06	34.56**	3.77**
QL	1		1.34*	0.00	0.75	0.03	0.96	0.76
QQ	1		2.99**	0.01	5.63*	1.42*	0.40	1.50
QC	1		0.73	0.01	4.11	3.21**	1.66	6.24**
CL	1		1.35*	3.14*	9.88**	0.08	1.44	2.44*
CQ	1		0.61	0.07	1.78	0.00	4.70	0.48
CC	1		2.90**	0.04	1.04	0.44	0.03	0.83
QL	1		0.09	5.95*	7.82*	2.26**	2.74	1.21
QQ	1		5.22**	1.84	9.18**	2.00**	0.08	1.57
QC	1		0.75	3.16*	0.79	0.69	8.98	0.52
Error	38	0.07	0.24	0.75	1.22	0.24	4.56	0.43
Total	59							
L. S. D.		0.44	0.81	1.43	1.83	0.81	3.53	1.09

Table 14. The effect of different proportions of salt and osmotic concentrations on the average dry weight of various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 0.3872

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.49	2.40	1.53	0.63	1.76
	2	3.19	2.74	1.71	0.92	2.14
	3	3.64	2.02	1.62	0.87	2.04
	4	3.32	2.38	1.27	1.16	2.03
	5	4.50	2.18	1.47	1.16	2.33
	$\bar{x}$	3.43	2.34	1.52	0.95	2.06

S. E. = 0.1732

S. E. = 0.1963

(b) Roots

S. E. = 0.15

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	0.83	0.97	0.71	0.27	0.70
	2	1.26	1.10	0.66	0.39	0.85
	3	1.25	0.77	0.67	0.38	0.77
	4	1.16	0.89	0.52	0.55	0.78
	5	1.65	0.72	0.55	0.42	0.84
	$\bar{x}$	1.23	0.89	0.62	0.40	0.79

S. E. = 0.0678

S. E. = 0.0761

Table 15. The effect of different proportions of salt and osmotic concentration on the uptake of sodium by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 0.6782

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	0.62	2.52	6.57	28.80	9.62
	2	0.35	1.92	3.53	8.60	3.60
	3	0.40	1.35	3.60	9.50	3.71
	4	0.29	0.83	2.07	4.73	1.98
	5	0.16	0.18	0.21	0.35	0.23
	$\bar{x}$	0.36	1.36	3.19	10.40	3.83

S. E. = 0.3047

S. E. = 0.3404

(b) Roots

S. E. = 0.283

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.87	8.63	10.60	8.00	7.52
	2	2.46	7.33	10.80	5.50	6.52
	3	1.47	4.72	6.50	5.00	4.42
	4	0.98	4.05	5.05	3.55	3.41
	5	0.41	0.27	0.52	0.35	0.39
	$\bar{x}$	1.64	5.00	6.69	4.48	4.45

S. E. = 0.1264

S. E. = 0.1414

Table 16. The effect of different proportions of salt and osmotic concentrations on the uptake of calcium by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 1.0104

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.57	4.53	1.67	3.60	3.09
	2	3.20	3.00	4.27	5.00	3.87
	3	4.13	3.80	4.72	9.03	5.42
	4	3.55	5.47	6.63	13.86	7.38
	5	4.65	7.30	9.98	16.27	9.55
	$\bar{x}$	3.62	4.82	5.45	9.55	5.85

S. E. = 0.4527

S. E. = 0.5059

(b) Roots

S. E. = 0.500

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.80	2.37	1.40	5.00	2.90
	2	1.87	1.77	2.90	5.80	3.08
	3	2.77	3.40	4.27	10.60	5.26
	4	2.10	1.48	3.80	8.27	3.91
	5	1.35	1.50	4.83	12.20	4.97
	$\bar{x}$	2.18	2.10	3.44	8.37	4.02

S. E. = 0.2236

S. E. = 0.250

Table 17. The effect of different proportions of salts and osmotic concentrations on the uptake of potassium by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 0.4722

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	7.87	6.50	3.87	2.40	5.16
	2	9.13	5.95	4.43	2.63	5.54
	3	7.80	5.40	4.27	4.93	5.60
	4	8.07	5.80	5.60	4.43	5.97
	5	7.97	5.17	4.33	4.93	5.60
	$\bar{x}$	8.17	5.76	4.50	3.87	5.57

S. E. = 0.2109

S. E. = 0.2362

(b) Roots

S. E. = 0.637

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	8.73	9.57	8.40	4.10	7.70
	2	10.20	9.87	11.60	6.70	9.59
	3	12.53	9.60	8.47	3.90	8.62
	4	12.50	13.20	11.83	4.00	10.38
	5	13.77	15.13	12.83	6.00	11.93
	$\bar{x}$	11.55	11.47	10.62	4.94	9.65

S. E. = 0.2846

S. E. = 0.3178

Table 18. The effect of different proportions of salt and osmotic concentrations on the uptake of magnesium by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 0.2886

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	3.00	3.43	2.70	3.23	3.09
	2	2.30	2.83	3.93	2.71	2.94
	3	2.40	2.13	2.47	3.30	2.57
	4	1.93	1.33	0.75	0.83	1.21
	5	2.00	0.73	0.53	0.40	0.92
	$\bar{x}$	2.33	2.09	2.08	2.09	2.15

S. E. = 0.1288

S. E. = 0.1449

(b) Roots

S. E. = 0.283

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	0.62	0.79	2.40	3.00	1.70
	2	0.97	1.57	1.60	2.50	1.66
	3	0.97	3.57	2.70	3.30	2.63
	4	1.23	0.85	0.70	0.60	0.87
	5	1.27	0.70	0.67	—	0.66
	$\bar{x}$	1.01	1.49	1.61	1.88	1.50

S. E. = 0.1264

S. E. = 0.1444

Table 19. The effect of different proportions of salt and osmotic concentrations on the uptake of chloride by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 2.6019

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	1.40	3.77	6.73	23.30	8.80
	2	1.50	3.82	7.23	11.07	5.90
	3	2.30	5.60	7.87	17.00	8.19
	4	1.10	5.23	8.77	23.60	9.67
	5	1.80	7.67	12.87	29.43	12.94
	$\bar{x}$	1.62	5.22	8.69	20.88	9.10

S. E. = 1.300

S. E. = 1.161

(b) Roots

S. E. = 1.2328

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	0.88	6.83	2.53	5.30	3.88
	2	1.30	6.80	2.47	4.57	3.78
	3	1.63	7.13	8.00	5.73	5.63
	4	1.93	9.57	8.67	5.33	6.37
	5	1.60	7.17	8.33	3.50	5.15
	$\bar{x}$	1.47	7.50	6.00	4.89	4.96

S. E. = 0.6164

S. E. = 0.5513



Table 20. The effect of different proportions of salt and osmotic concentrations on the uptake of phosphorus by various parts of Hubam clover and their standard errors

(a) Leaves and stem

S. E. = 0.5916

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	2.94	2.61	3.35	2.64	2.88
	2	3.35	3.01	2.98	3.75	3.27
	3	2.36	3.07	3.14	3.34	2.98
	4	2.31	2.73	2.92	4.87	3.21
	5	2.40	2.65	2.66	2.86	2.64
	$\bar{x}$	2.67	2.82	3.01	3.49	3.00

S. E. = 0.2645

S. E. = 0.2958

(b) Roots

S. E. = 0.3741

		Osmotic pressure				
		1	3	6	9	$\bar{x}$
Treatments	1	5.17	5.17	8.24	5.10	5.92
	2	5.95	6.17	8.24	6.28	6.66
	3	5.15	5.78	6.80	9.32	6.76
	4	4.80	5.60	7.13	9.65	6.80
	5	5.93	5.58	8.53	10.31	7.59
	$\bar{x}$	5.40	5.66	7.79	8.13	6.75

S. E. = 0.1688

S. E. = 0.1892