GROWTH AND BIOCHEMICAL COMPOSITION OF BEAN PLANTS AS CONDITIONED BY SOIL MOISTURE TENSION AND SALT CONCENTRATION

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(WITH NINE FIGURES)

There is evidence in the literature that decreasing soil moisture content is associated with increases in the osmotic pressure of the tissue fluids in both roots and tops of plants (**26, 27**), decreases in rate of vegetative growth (**1, 9, 15, 30, 64**), modifications in stomatal opening (**16, 17**), a depletion in starch reserves (**15, 35, 52, 64**), a decrease in apparent photosynthesis, and an increase in respiration (**50**). Many of these effects were noted even though the respective plants did not wilt, and notwithstanding the fact that there is considerable evidence (**8, 24, 58, 62**) that all soil moisture above the wilting percentage (that held by less than 15–18 atmospheres of tension) is equally available to plants, and that variations in this range of available moisture are without effect on plant growth.

Most of the foregoing observations were made on non-saline soils. There appears to be little information available as to the effect of increasing salt concentration in the soil solution upon plant metabolism. The best work bearing on this relationship is that of NIGHTINGALE and FARNHAM (**37**). They grew sweet peas in sand culture with complete nutrient solutions varying in osmotic pressure with the purpose of inducing "physiological drought." They presented their data on the green weight basis and found that the content of sugars and starch in the plants increased with an increase in the osmotic pressure of the substrate. By contrast, both the soluble organic nitrogen and the protein contents of the plants decreased, even though there were ample amounts of nitrate nitrogen present in the tissues. These authors suggested that "protein starvation" was actually involved in the reductions in meristematic activity and in vegetative growth accompanying increased concentration of the culture solution.

WADLEIGH and GAUCH (**63**) grew Red Kidney beans in water culture in which high concentrations of $CaCl_2$ and NaCl were added to the base nutrient and assayed the plants for certain nitrogenous and carbohydrate constituents. They found a progressive diminution in the concentration of NO_3–N in the tissues with increase in osmotic pressure of the substrate in the presence of either salt. Percentage of protein, especially in the leaves, also decreased with increasing salt concentration, whereas percentage of soluble organic N was practically unaffected. The plants were grown at a time of year (December) when the daily quantity of solar radiation was low and, as a consequence, the carbohydrate level in the plants was relatively low. However, increasing the salt concentration of the culture medium was associated with general increases in both reducing and nonreducing sugars in all parts of the plants (leaves, stems, and roots). None of the plants subjected to saline treatments showed any definite starch accumulation above that of the control plants. Those grown on the lower concentrations of added salt were found to have a definitely lower percentage of starch than the controls; but, within saline cultures, percentage of starch tended to increase with increased osmotic pressure of the culture solution. In comparing the results of these two investigations it should be stressed that NIGHTINGALE and FARNHAM (**37**) increased the osmotic pressure of their nutrient solutions by uniformly increasing all constituents, thereby effecting a large increase in nitrate supply; whereas WADLEIGH and GAUCH (**63**) maintained the nitrate level constant and increased the osmotic pressure by additions of chloride salts only. In the latter study, the high concentrations of the chloride ion may have been somewhat inhibitory to carbohydrate synthesis. Heavy applications of chloride salts in fertilizers have been found to decrease the chlorophyll content of potato leaves (**5, 41**) with a concomitant decrease in the total carbohydrate content of the leaves (**4**).

A previous report (**3**) from this laboratory presented data on the yield of Red Kidney bean plants subjected to varying degrees of salt concentration and soil moisture tension. The wide range in yields observed was regarded as indicative of a similar range in the metabolic status of the plants during the period of active vegetative growth.

It follows that the effect of soil salinity upon crop yields can be better interpreted with the advent of increasing information concerning the effect of saline substrates on specific metabolic processes within the plant. Consequently, the above-mentioned experiment (**3**) was repeated with the exception that the plants were harvested at incipient florescence and assayed for a few of the biochemical constituents important in plant metabolism.

Experimental procedure

Thirty-six steel drums with a ten-gallon capacity were each filled with Fallbrook loam soil. Determinations on a pressure-membrane apparatus (**46**) indicated that this soil held 6.2 per cent. moisture when moistened and allowed to come to equilibrium under a tension of 15 atmospheres. The permanent wilting percentage determined with sunflower plants was 6.1. The close agreement of these two values corroborates the report by RICHARDS and WEAVER (**46**) that the 15-atmosphere percentage lies in the wilting range. The moisture equivalent of this soil was 14.7 per cent., and the normal moisture capacity 18.0 per cent. (**51**). The tension on the moisture in this soil as a function of percentage of moisture is shown in figure 1. With the moisture content at saturation, the original soil had a pH of 7.6 and a specific conductance of 34×10^{-5} mhos at 25° C. Calcium was the predominating cation in the exchange complex at the start of the experiment, and there were no accumulated soluble salts in the original soil.

The authors are indebted to DR. MILTON FIREMAN for the data on the tension on the moisture in soil as a function of percentage of moisture; such data were secured by methods described elsewhere (**42, 45**). These data are

frequently plotted with a logarithmic scale (log tension in centimeters of water) as the ordinate rather than with the linear scale used in figure 1. The latter presentation emphasizes the hyperbolic nature of this curve; *i.e.*, it accentuates the fact that as moisture content decreased in this soil from 30 to 11 per cent. the tension increased only about one atmosphere; whereas a drop in soil moisture content from 11 to 6 per cent. caused the tension to increase by 14 atmospheres.

The experimental variables were:

I. Salt treatments 4
- a. No added NaCl
- b. 0.1 per cent. added NaCl on dry soil basis
- c. 0.2 per cent. added NaCl on dry soil basis
- d. 0.4 per cent. added NaCl on dry soil basis

II. Irrigation schedules 3
- a. Irrigated when only 40–50 per cent. of the available moisture had been removed from the soil
- b. Irrigated when 60–65 per cent. of the available moisture had been removed from the soil
- c. Irrigated when 90–100 per cent. of the available moisture had been removed from the soil

The drums were randomized within 3 blocks. This gave one drum of each combination of salt and irrigation schedule per block. Following established convention, the available moisture in a soil was taken as that between the field capacity (20 per cent.) and the permanent wilting percentage.

The proper amount of salt for each treatment was mixed with the screened soil ($\frac{1}{4}''$) in a box suspended and rotated eccentrically. Besides the designated sodium chloride, 3.4 grams of potassium chloride and 10.1 grams of 16 per cent. superphosphate were added to each drum of soil. One hundred and seven pounds of the soil at a moisture content of 5 per cent. were placed in each container with the aid of a mechanical mixer and packer. Nitrate was supplied by scratching 13.2 grams of calcium nitrate dihydrate into the soil surface of each drum just before the initial irrigation. These amounts of fertilizing materials corresponded to an application of 1000 pounds of 6–8–6 fertilizer per acre (per 2,000,000 pounds of soil).

Tensiometer cups (**43**) were placed at depths of 4 inches and at 14 inches (bottom) in each drum of one set of replicates. Enough tap water was added to bring the soil to an average moisture content of 20 per cent. This value was identical with that found for the field capacity of this soil. The drums were weighed daily and when the average moisture content reached a designated degree of depletion sufficient tap water was added to reestablish the 20 per cent. level of soil moisture.

On April 4, 1942, after the irrigated soils had been standing for several days, eight germinated dwarf Red Kidney beans were placed in each culture. When the seedlings were well established, they were thinned to the four most uniform per container. The plants were grown until the first blossoms were beginning to appear and then harvested. This harvest took place between 6:00 and 7:30 A.M. on May 7. Plants in all treatments were turgid at this time. The sky was heavily overcast at this time and a mini-

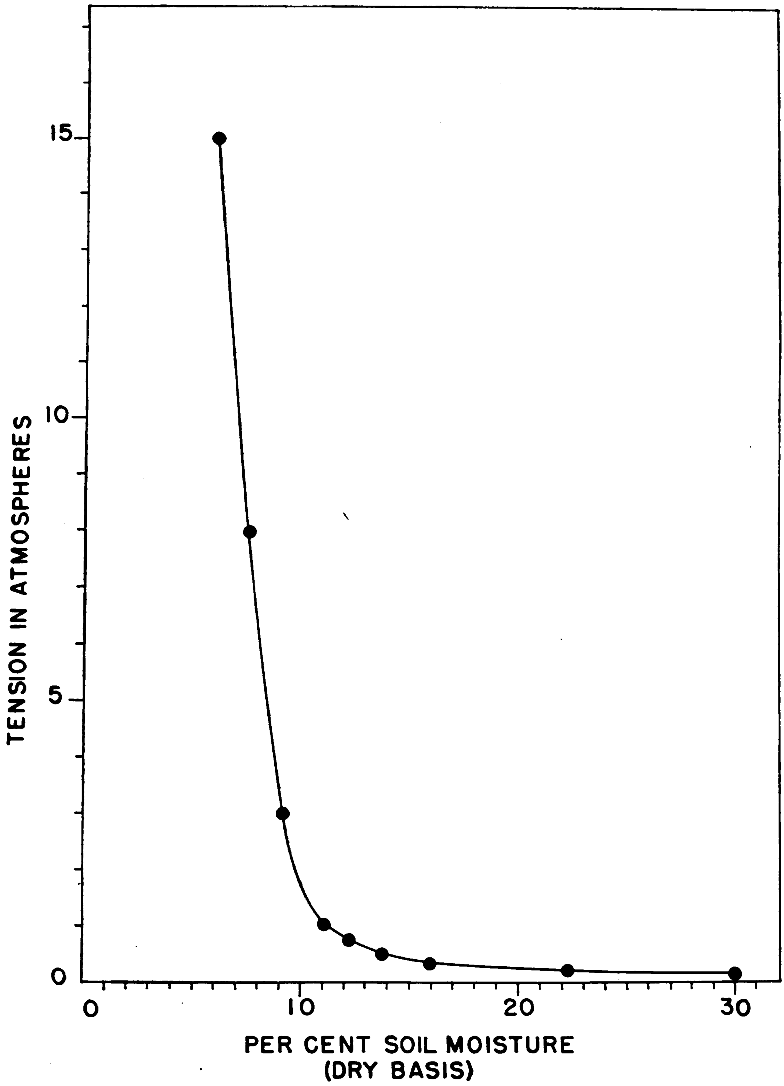

FIG. 1. Relationship between soil moisture percentage and moisture tension of the sample of Fallbrook loam.

mum of change in the relative status of the metabolites was regarded to have taken place during this time interval. On harvesting, each plant was weighed, segregated into (a) leaves, and (b), stems plus petioles, and prepared for preservation by rapid desiccation in a forced draft oven maintained at about 75° C. The dried tissue was ground in a small Wiley mill and then in a ball mill until all tissue would pass a 100-mesh screen.

Total nitrogen including nitrate was determined by a micro-modification of the method of RANKER (**40**). Total soluble nitrogen was extracted according to the procedure described by Clark (**7**), and assayed by the method of PUCHER and VICKERY (**39**) using reduced iron to effect nitrate reduction. Nitrate nitrogen in the extract was determined by a micro-modification of the usual DEVARDA method. Soluble organic nitrogen was taken as the difference between nitrate and total soluble nitrogen. "Protein" nitrogen was calculated as the difference between total nitrogen and total soluble nitrogen.

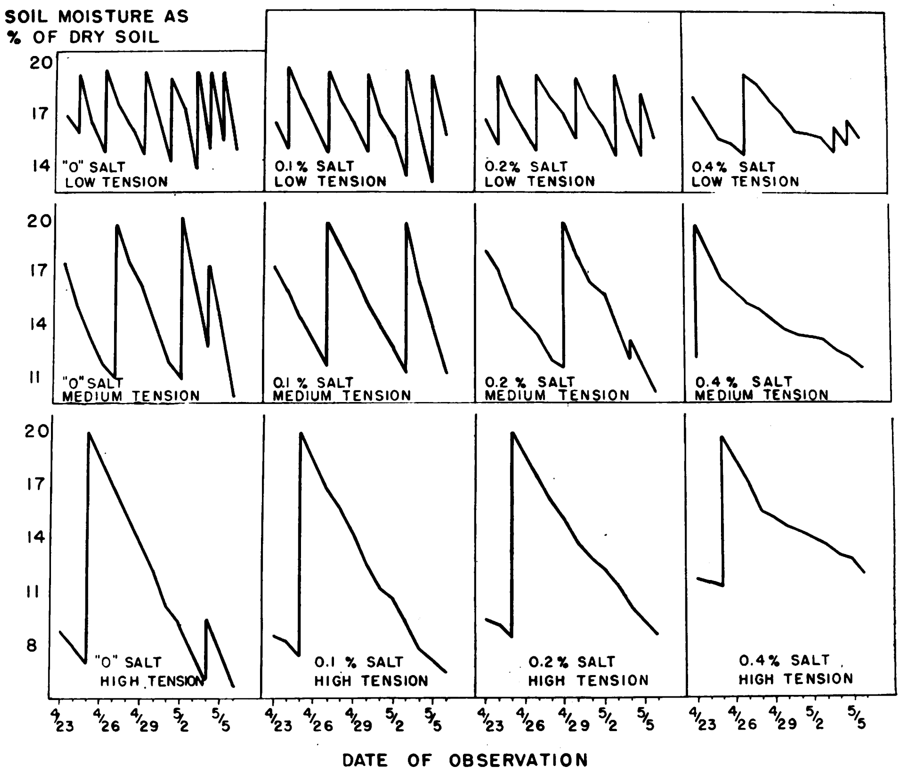

FIG. 2. The range of average soil moisture percentage in the soil of the various treatments for two weeks prior to the harvest.

For sugar determinations, 500 mg. of the powdered tissue was extracted with 100 ml. of 85 per cent. alcohol by refluxing one-half hour. After removing the alcohol from the extract, clearing, and deleading, the reducing and non-reducing sugars were determined by the method of TOMPSETT (**55**). Invertase was used as the hydrolytic agent for sucrose. Starch was determined on the residue following extraction of dextrins with 10 per cent. alcohol by the method described by LOOMIS and SHULL (**31**) using fresh saliva as the hydrolytic agent.

Results

Figure 2 presents the trends of average soil moisture content for each treatment during the last two weeks of the experimental period. The plants on the "0" salt-"low"-moisture-tension treatment had to be watered nearly every day during the last few days of the study. As the salt content of the soil was increased the frequency of irrigation within a given soil moisture regime decreased. This was due to the fact that higher levels of soil salinity resulted in a corresponding reduction in plant growth, thereby lowering the rate of water removal from the soil. As the degree of soil moisture depletion prior to irrigation was intensified, the frequency of irrigation was also reduced. This reduction in irrigation-frequency was not entirely due

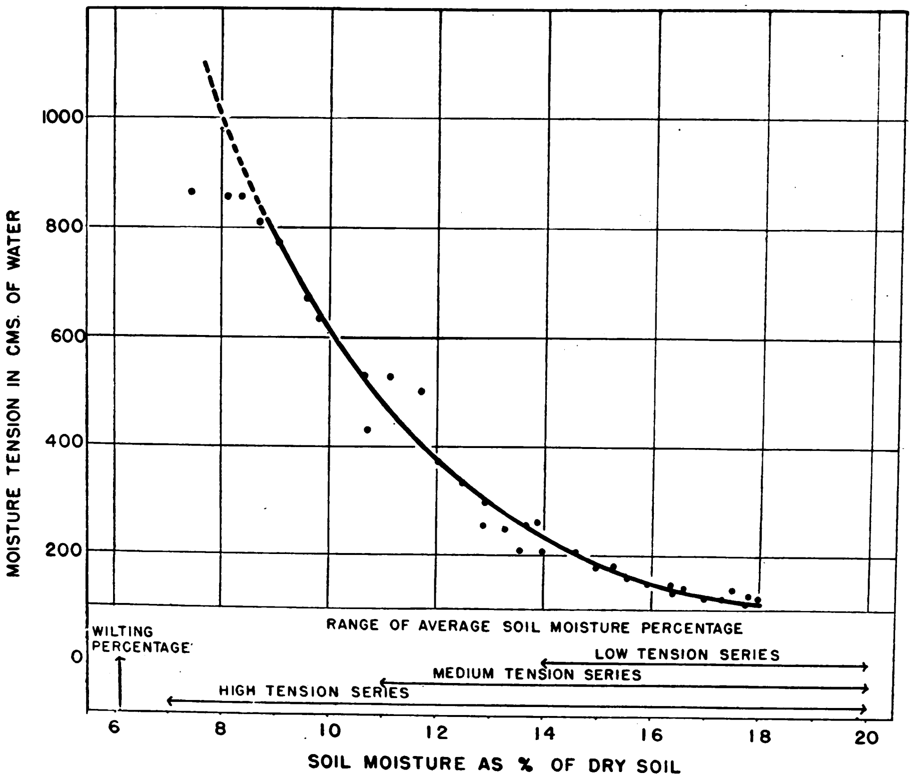

FIG. 3. The relationship between the average soil moisture of the "0" salt treatment and the tension at the four-inch depth.

to the wider ranges in soil moisture content, since the correlated reduction in plant growth resulted in decreased rate of water removal. It was planned that the date of harvest should occur when the soil moisture-tension would be approaching the maximum specified for any given treatment. This goal was only partially realized. For example, the moisture levels of the "medium-tension" and "high-tension" treatments in the soil containing 0.4 per cent. salt were so nearly identical prior to the date of harvest that little or no response to differential treatment could be expected.

It may be seen from figure 3 that tensiometer readings (at the 4-inch depth) for various average soil moisture contents in the "0" salt treatments tended to follow the general trend of that shown in figure 1, but with an appreciable displacement to the left. Tensiometers are not reliable above 800 cm. of tension, thereby accounting for the anomalous observations at the lower moisture percentages. RICHARDS and FIREMAN (**45**) have shown that the locus of a soil moisture sorption curve may be shifted depending on treatment of the soil prior to the determination. Furthermore, it should be emphasized that the soil moisture percentages used in figure 3 are averages for the whole mass of the 100 pounds of soil, whereas the tensiometer values indicate the moisture status of only a portion of the total soil mass in the drum. That is, one could not expect the loci of the respective curves in figures 1 and 3 to coincide.

The average green and dry weights per plant produced under the imposed experimental conditions are shown in table I and the former in

TABLE I

AVERAGE GREEN AND DRY WEIGHTS PER PLANT FROM THE VARIOUS TREATMENTS

TREATMENT		GREEN WT.	DRY MATTER	DRY WT.
SALT LEVEL	MOISTURE TENSION			
%		gm.	%	gm.
"0"	Low	61.0 ± 2.8*	14.1 ± 0.11	8.62 ± 0.32
"	Med.	51.3 ± 4.1*	13.6 ± 0.09	7.42 ± 0.58
"	High	32.5 ± 1.2	15.8 ± 0.08	5.17 ± 0.20
0.1	Low	46.2 ± 0.8	13.6 ± 0.01	6.30 ± 0.11
"	Med.	35.5 ± 2.1	14.0 ± 0.19	4.95 ± 0.34
"	High	23.7 ± 1.0	15.5 ± 0.08	3.67 ± 0.17
0.2	Low	30.7 ± 2.6	13.9 ± 0.12	4.27 ± 0.38
"	Med.	24.1 ± 0.5	14.7 ± 0.05	3.67 ± 0.05
"	High	18.1 ± 0.2	14.9 ± 0.11	2.70 ± 0.02
0.4	Low	9.6 ± 0.4	14.8 ± 0.23	1.42 ± 0.07
"	Med.	8.4 ± 0.8	14.9 ± 0.08	1.27 ± 0.13
"	High	7.7 ± 0.8	14.8 ± 0.05	1.12 ± 0.10

* All errors given are standard errors.

figure 4. The observed trends not only show the marked reduction in growth resulting from increased soil salinity, but also indicate that the level of response to a given salt content of the soil was modified by the extent of soil moisture depletion prior to irrigation. To be sure, the concentration of the soil solution will increase as the soil dries out but, in a non-saline soil, the pressure potential becomes a more important component of the free energy of the soil moisture than the osmotic potential (**10**). The above responses towards increasing soil moisture tension confirm those previously reported (**3**) for a parallel experiment in which yields of dried beans were used as the criterion of growth.

The term "pressure potential" is sometimes designated as "capillary potential." The moisture potential (**61**) of the soil moisture (free energy per unit mass) was shown by DAY (**10**) to be the summation of (a) the pressure potential (free energy per unit mass developed by attractive forces between soil particles and surrounding water), and (b) the osmotic potential (free energy per unit mass due to solutes). Tensiometers and pressure-membrane apparatus measure the pressure potential of the soil moisture; the osmotic potential of the displaced soil solution may be determined cryoscopically. A potential function is not dimensionally expressible in terms

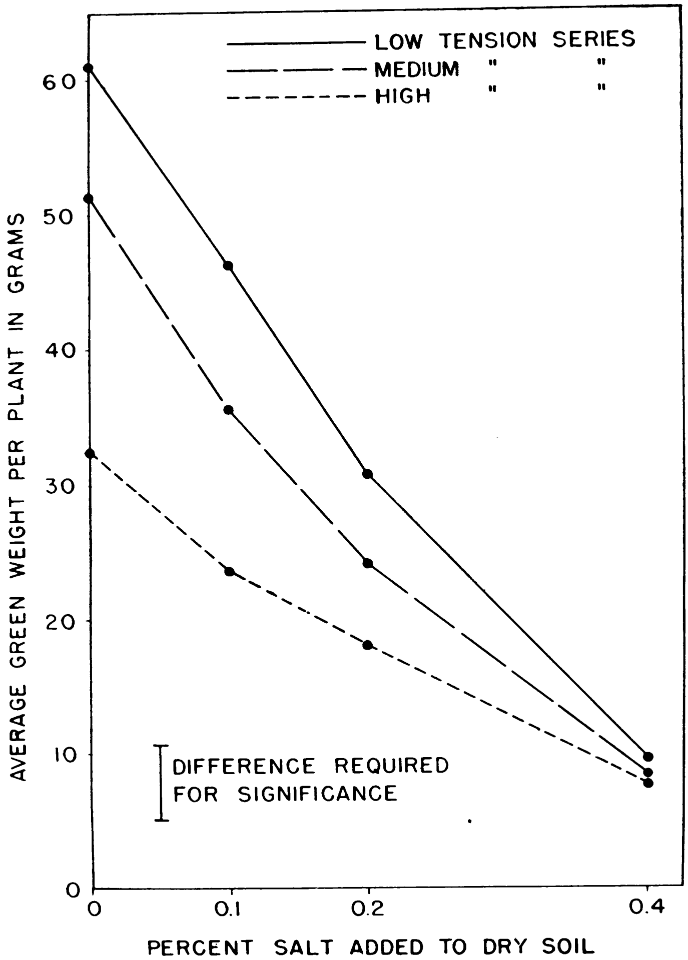

FIG. 4. Average fresh weight of plants.

of atmospheres, but it is conducive to clarity in this report to discuss the forces acting on the soil moisture in terms of atmospheres. A moisture potential of -1×10^6 ergs per gram is approximately equivalent to a pressure of one atmosphere. In line with the work of RICHARDS and WEAVER (**46**), the term "moisture tension" expressed in atmospheres will be used to designate the free energy of the soil moisture involved in the pressure potential, osmotic pressure to designate that of the osmotic potential, and "total moisture stress'' in atmospheres (sum of osmotic pressure + moisture tension) will be used to designate the total free energy of the soil moisture as covered by the term moisture potential.

The variations in percentage of dry matter found in these plants are shown in figure 5. Under the water regimes designated as ''low'' and ''medium'' tension, there was a tendency for the percentage of dry matter to increase with increase in salt content of the substrates, excepting that

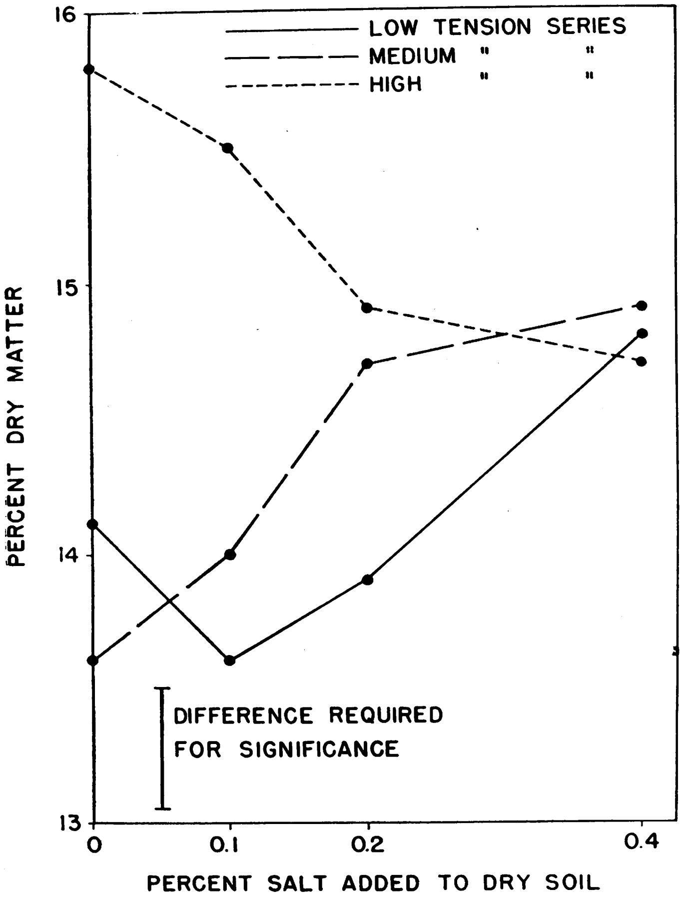

FIG. 5. Average percentage dry matter of plants.

within the ''low'' tension series the ''0'' salt plants had a significantly higher percentage of dry matter than those grown on soil containing 0.1 per cent. salt. In contrast, the plants in the ''high'' tension series showed a definite decrease in percentage of dry matter with increased soil salinity. This is not an unusual finding; another study (**64**) on beans grown in saline soil consistently showed this same trend. In general, the relative positions of the curves in figure 5 tend to show an increased percentage of dry matter with increased degree of soil moisture depletion prior to irrigation, even

TABLE II

BIOCHEMICAL COMPOSITION OF BEAN LEAVES AND STEMS AS PERCENTAGE OF DRY MATTER

PLANT PART	TREATMENT		NO_3–N	SOL. ORG. N	PROTEIN N	TOTAL N	REDUCING SUGARS	NON-REDUCING SUGARS	STARCH	HEMI-RESERVES
	SALT LEVEL	MOISTURE TENSION								
	%		%	%	%	%	%	%	%	%
Leaves	"0"	Low	0.08	0.47	2.94	3.51	0.97	2.46	4.64	5.90
	"	Med.	0.17	0.49	3.50	4.16	0.67	1.46	0.59	5.34
	"	High	0.25	0.61	3.63	4.49	0.81	1.30	0.59	5.18
	0.1	Low	0.08	0.39	3.40	3.77	0.78	1.61	2.76	6.22
	"	Med.	0.13	0.54	3.42	4.09	0.77	1.63	1.29	5.64
	"	High	0.16	0.67	3.58	4.41	0.70	1.65	0.42	5.60
	0.2	Low	0.13	0.49	3.66	4.28	0.45	1.39	2.38	6.08
	"	Med.	0.18	0.59	3.95	4.72	0.47	1.44	0.66	5.80
	"	High	0.17	0.61	3.89	4.50	0.53	1.65	0.60	5.94
	0.4	Low	0.23	0.62	3.82	4.44	0.84	1.23	0.87	5.68
	"	Med.	0.23	0.61	4.09	4.70	0.63	1.36	0.41	5.06
	"	High	0.25	0.67	4.13	4.80	0.78	1.33	0.44	6.02
Stems	"0"	Low	0.12	0.71	1.19	2.02	4.74	3.63	2.12	12.58
	"	Med.	0.20	0.82	1.34	2.36	4.30	3.49	1.39	11.32
	"	High	0.40	0.97	1.72	2.69	3.40	2.08	1.40	8.92
	0.1	Low	0.10	0.71	1.31	2.02	3.06	2.94	1.35	12.96
	"	Med.	0.23	0.78	1.54	2.32	3.25	2.58	1.06	11.04
	"	High	0.37	1.00	1.70	2.70	2.73	2.35	1.70	13.14
	0.2	Low	0.29	0.82	1.62	2.44	2.54	2.09	1.08	12.58
	"	Med.	0.36	0.93	1.61	2.52	2.62	2.36	1.02	13.34
	"	High	0.37	0.93	1.59	2.52	2.29	2.28	1.12	13.34
	0.4	Low	0.48	0.97	1.73	2.70	2.19	2.35	0.80	12.92
	"	Med.	0.50	0.97	1.86	2.83	2.03	2.18	1.13	12.19
	"	High	0.58	1.05	1.93	2.98	1.64	1.90	0.90	12.80

though soil moisture regime is without effect at the highest salt level. The seemingly anomalous positions of the "low" and "medium" tension observations at "0" salt level cannot be adequately explained, and are probably merely fortuitous. It was even somewhat surprising that the "low" and "medium" tension curves showed a positive trend, since it has been observed (**5**, **22**) that increasing concentrations of chlorides in the substrate effect

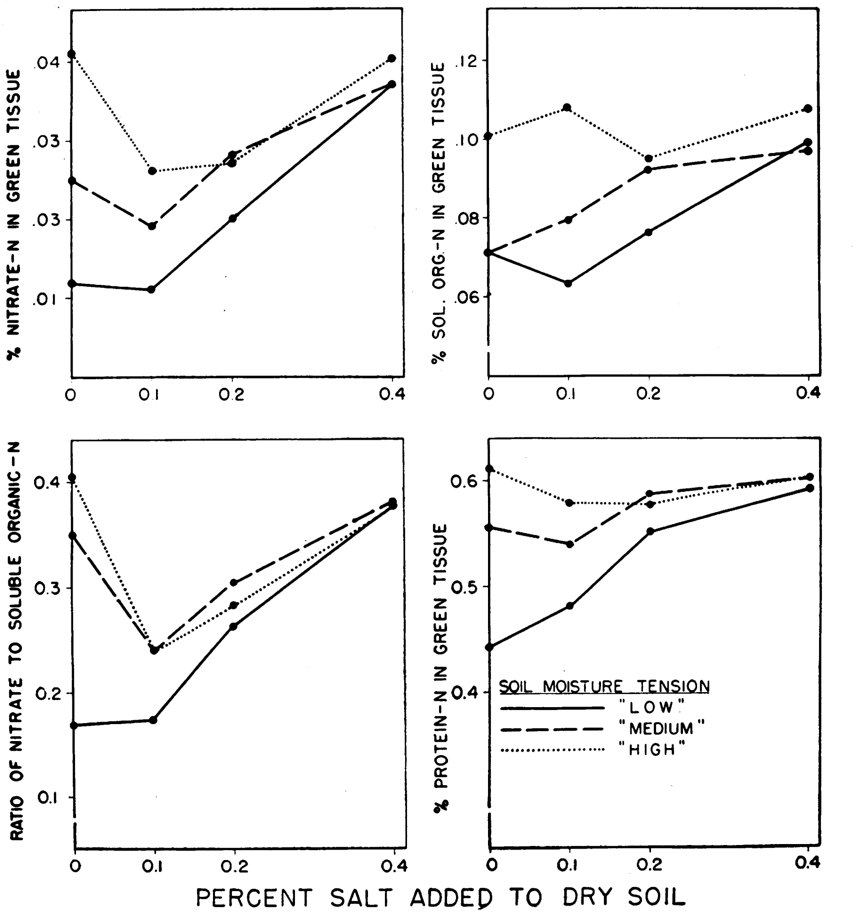

FIG. 6. Trends in the nitrogenous constituents in the leaves of the experimental plants.

either no change or an actual decrease in the percentage dry matter of plants.

Table II shows the percentage composition of various nitrogenous and carbohydrate constituents of the dry matter of the leaves and stems of these plants. Figure 6 shows the trends of the major nitrogen fractions found in the leaves on the green weight basis. Nitrate-N and soluble-organic-N found in the stems showed similar trends, whereas the "protein" content of stems showed practically no variation.

The level of nitrate nitrogen in the plants provides an index of the differential between the rate of nitrogen absorption and the rate of nitrogen assimilation. This fraction is especially pertinent in this study in that the total nitrogen of plants from all treatments indicated that they should be characterized as "high nitrogen" plants. There is a decided trend for percentage of NO_3-N to increase with increased salt content of the substrate; and, also, for this percentage to increase with increase in degree of soil desiccation prior to irrigation. With few exceptions the differences between results from treatments having "0" and 0.1 per cent. added salt deviate from the general trend. This may be fortuitous, but LONG (**29**) has shown that when appreciable quantities of NaCl are present in the substrate, absorption of NO_3-N is reduced. The observed results suggest that the first increment of salt inhibited rate of absorption relatively more than rate of assimilation; but at high intensities of soil salinity, this intial effect on the differential between the two rates was moderated or even reversed.

The status of soluble organic nitrogen is usually an index of the level of nitrogen metabolism within plants. The trends of this component within the leaves are also shown in figure 6. The relative loci of these curves present a pattern approaching that observed for NO_3-N. At "low" and "medium" tensions, increase in soil salinity was associated with an increased content of soluble organic nitrogen in the leaves. The trend under "high" tension conditions tended to be the reverse. It is also evident (fig. 6) that there was a marked trend for the ratios of nitrate/soluble-organic-N to increase with increase in salt content of the soil, indicating that NO_3-N was absorbed faster than it was assimilated at the higher levels of soil salinity. In leaves from plants at higher moisture tensions, the first increment of added salt showed a reversal of this general trend.

Since the soluble organic nitrogen fraction is largely composed of amides, amino acids, and peptides—"building blocks" for proteins—it might be expected that the trends for the protein nitrogen content of these plants would line up correspondingly to those found for soluble organic nitrogen. This was found to be the case for the leaves but there was practically no variability in percentage protein in the stems. It appears that increases in both the osmotic pressure and the moisture tension of the soil moisture tended to increase the "protein" reserves in the leaves, but that when either variable was at its maximum stress the effect of the other was practically eliminated. Although the absolute range in these values for percentage of protein appears small, they are relatively large for leaves similar in age.

It is evident that the experimental treatments had a definite effect upon the status of nitrogen metabolism in these plants. But, what is more important, there was no evidence that nitrogen assimilation was inhibited to the extent of limiting growth. On the contrary, the highly important proteinaceous reserves tended to accumulate under the more intense conditions of stress.

One cannot fully interpret the nitrogen metabolism of plants without cognizance of the status of carbohydrate constituents. In contrast to the similarity in relationships observed for the various nitrogenous fractions, it was found that various carbohydrate constituents showed certain differences in the relative trends incurred by the experimental conditions. The analytical results are presented in table II and partially in figure 7.

There were no significant differences in percentages of reducing sugars in the leaves, those from all treatments being low in this fraction. Bean

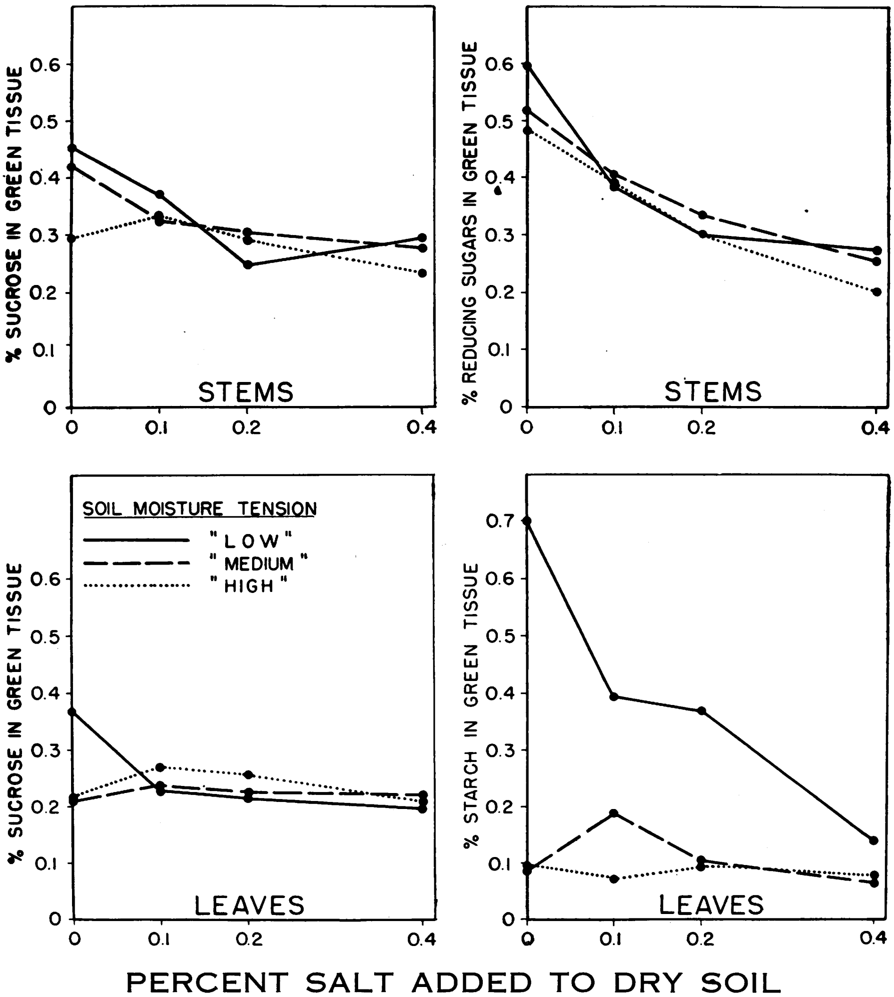

FIG. 7. Trends in the carbohydrate constituents of the experimental plants.

stems have a higher percentage of reducing sugars than the leaves, and soil salinity had a marked effect in decreasing the percentage of these sugars in the stems. Intensity of soil moisture tension appeared to have no effect on the level of these sugars. The parallelism noted for the effects of moisture tension and osmotic pressure of the soil moisture with respect to nitrogen metabolism is missing from the picture for reducing sugars. It is possible that these trends reflect the specific effect of the chloride ion in lowering photosynthetic activity which has been noted for potatoes (**5**, **41**) even though this effect was noted in the stems rather than the leaves. The results reported are at variance with those (**37**, **63**) showing accumulations of reducing sugars in plants grown in artificial cultures of increasing salt concentration, and suggest that environmental conditions modify the effect of salinity on the status of reducing sugars in plants. The lowest percentages observed for this carbohydrate in no sense indicate a serious depletion with respect to affecting growth since vigorously vegetative bean stems may contain a considerably lower percentage of reducing sugars (**63**).

Variations in salt content of the soil had relatively little effect upon the percentage of nonreducing sugars in either leaves or stems, even though there was a slight tendency for percentage of sucrose to decrease with increase in soil salinity (fig. 7). Other studies on beans (**63**), sweet peas (**37**), and barley (**20**) grown on sand cultures presented evidence that saline substrates induce marked accumulation of nonreducing sugars. In fact, these sugars were comparatively abundant in plants of all treatments of the present study. In the cultures of the non-saline soil, the lower the soil moisture tension was maintained, the higher the percentage of "sucrose" in both stems and leaves. It appears that soil moisture tension and salt concentration of the soil solution had somewhat similar effects on the concentration of nonreducing sugars in these plants.

With respect to the starch reserves in the leaves of these plants, figure 7 shows that increasing either soil moisture tension or salt concentration of the soil had a marked effect. Variations in the starch content of the stems were negligible. At the higher levels of soil moisture tension, the starch content of the leaves was low and the salinity of the soil had little effect on the percentage of this constituent. This is in agreement with the results of another study with beans grown on saline soil (**64**) in which it was found that starch reserves were markedly depleted by soil desiccation even though the plants did not wilt, and the starch reserves were redeveloped within 24 hours after irrigating the desiccated soil. MAGNESS *et al.* (**35**) have found that soil moisture depletion induces a lowering of the starch reserves in apple trees. These observations are in accord with the findings of SPOEHR and MILNER (**52**) to the effect that moisture stresses within plants cause increased amylolytic activity and starch dissolution. Yet, it is of interest that the high percentage of starch in the leaves of the "0" salt, low-tension plants, as compared with the starch content of leaves from other treatments, was not compensated by any tendency for sugars to accumulate in the plants of these other treatments.

There is evidence (**47**, **57**) that subjecting plants to conditions approaching drought causes a marked increase in the content of hemicelluloses in the tissues. These constituents have been considered important in the drought resistance of plants. Variation in the water relations of these bean plants induced by the experimental conditions had no definite effect on the status of hemi-reserves within the plants.

Discussion

Two of the main requirements for plant growth are the formation of protoplasm for new cells, and the hydration of that protoplasm together with vacuolation to bring about cell enlargement (**33**). The term "hydration" is here used in the same sense as implied by MacDougal (**33**); *i.e.*, imbibition rather than formation of stoichiometric hydrates. It has been shown that the conditions of stress, which brought about marked growth reduction, actually caused an increase in the proportion of proteinaceous material in the tissues, especially in the leaves. This increase occurred even though the plants showing reduced rates of growth would have a comparatively small

TABLE III

Osmotic pressure of the soil solution in the four different salt treatments as influenced by soil moisture percentages [from Ayers *et al.* (1)]

Added NaCl	Depth	Calculated* osmotic pressure† of soil solution at four soil moisture percentages			
		7%	11%	15%	20%
%	*in.*	*atmos.*	*atmos.*	*atmos.*	*atmos.*
0.0	0–5	1.67	1.07	0.78	0.59
0.0	5–10	4.26	2.71	1.99	1.49
0.0	10–15	5.59	3.56	2.61	1.96
0.1	0–5	4.41	2.81	2.06	1.55
0.1	5–10	12.6	7.99	5.86	4.40
0.1	10–15	25.7	16.3	12.0	8.99
0.2	0–5	9.80	6.23	4.57	3.43
0.2	5–10	20.5	13.1	9.57	7.18
0.2	10–15	42.7	27.2	19.9	14.9
0.4	0–5	18.8	12.0	8.77	6.58
0.4	5–10	34.0	21.7	15.9	11.9
0.4	10–15	86.0	54.7	40.1	30.1

* Values calculated assuming only simple dilution or concentration.

† Osmotic pressures of soil solutions were determined on displaced solutions from soils adjusted to approximately 12 per cent. moisture.

proportion of young cells containing abundant protoplasm (**22, 37**); the type of cells capable of synthesizing proteins from nitrate. Nightingale and Farnham (**37**) drew the inference that this histological situation together with the *relatively* low percentage of protein nitrogen they observed, resulted in a condition of "protein deficiency" with respect to vegetative growth. It has been suggested (**32, 38**) that under conditions of stress inhibitory to growth, certain phases of protein synthesis may become limiting; *i.e.*, the total quantity of protein present gives no information on the quality of that protein or the prevalence of types specifically essential for growth. It was found, however (**64**), that beans almost completely resumed normal rate of growth within 24 hours after the alleviation of stresses such as those under discussion. This was especially shown in unpublished data on the daily rate of growth of expanding leaves and suggests that inability to form new protoplasm is not a primary growth restrictive under the conditions of the present study. The data indicate that there were adequate proteinaceous and other nitrogenous reserves for a higher rate of cell formation than was taking place in the stunted plants. Consequently, limited swelling of the protoplasmic proteins and decreased vacuolation of the protoplasm were undoubtedly main factors inhibiting growth whether the water stress was brought about by high soil moisture tension or by high osmotic pressure of the soil solution.

An indication of the range in osmotic pressure of the soil solution for various soil moisture percentages in the four different salt treatments is given in table III. These determinations were made on the same soil identically treated in a parallel experiment (**3**). These data together with those given in figure 1 show the extent of the decreases in free energy of the soil moisture which were induced by increasing osmotic pressure on the one hand, or increasing tension (fig. 1) on the other. Thus, under conditions of maintained low soil moisture tension (average soil moisture varied between 14 and 20 per cent.) the osmotic pressure in the surface horizon increased from about 0.8 to 9.0 atmospheres as the percentage of added NaCl increased from 0 to 0.4 per cent. Correspondingly, when the osmotic pressure was maintained at a low level (''0'' added salt), depletion of the soil moisture caused an increase in the moisture tension from 0.3 to 12–15 atmospheres. In other words, decreasing the free energy of the soil moisture, whether by increasing moisture tension or by increasing osmotic pressure, would necessitate an increase in the diffusion pressure deficit within the plant in order to effect the ''passive'' entry of water into the roots. As a result, the free energy of the water in the plant tissues would also become lower. Decrease in the free energy of the dispersing medium is associated with a lowered degree of imbibition (swelling) of dispersed proteins (**49**). These facts support the conclusion that decreased imbibition by lyophilic colloids in developing cells is a factor limiting growth under the prevailing experimental conditions. Furthermore, a decrease in the free energy of the solution external to a cell would increase the osmotic work which would have to be done in bringing about vacuolation of the cytoplasm, thus inhibiting this phase of cell enlargement. Thut and Loomis (**54**) observed the diurnal growth of plants in relation to diurnal temperature, light, and humidity. They state that growth was checked by water deficits within the plant, and that such deficits were usually proportional to light intensity, temperature and air movement, and inversely proportional to relative humidity and the available soil moisture. The prevailing environmental conditions of the present experiment (*vide infra*) would certainly tend to accentuate the effect of soil moisture availability in growth.

Edlefsen (**14**) has suggested the elimination of most of the numerous and somewhat confusing terms dealing with water relations of plants. In their stead, he proposed evaluating the whole system in terms of free energy. This is a worthwhile motive. This concept has been especially valuable in studying the precise status of water in the substrate, but the complexities of plant processes which still reside in the realm of the unknown are not conducive to thermodynamic evaluation in quantitative terms. The plant system is seldom in equilibrium.

In the light of the foregoing, growth response of the plants should be the net effect through time of water stress induced both by solutes and by mois-

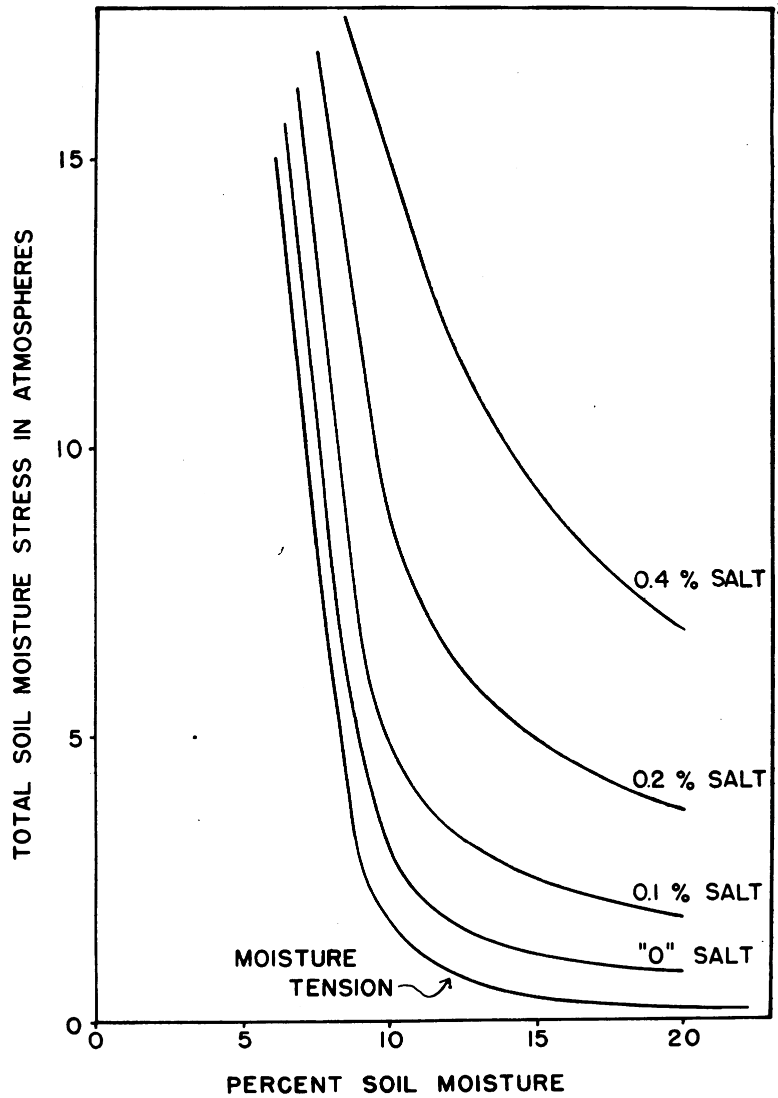

FIG. 8. The relation of moisture stress to moisture percentage at the different levels of soil salinity.

ture tension. Figure 8 indicates the minimum moisture stress to which the roots were subject at the various average soil moisture percentages. The curves for the various "salt levels" were derived by adding the osmotic pressure of the soil solution at the different average soil moisture percentages to the respective values for the moisture tension. The observations for osmotic pressure employed were those found for the surface horizon of the soil—that stratum of the soil containing the least solutes. This was considered the most valid approach since it is shown in water cultures (**11, 29**) that the greater proportion of water taken up by roots is from the portion of the substrate having the lower osmotic pressure. The trend of moisture stresses found for the "0" salt treatments were not greatly different from that of the soil moisture tension, indicating that the increase in osmotic pressure of the soil solution due to soil moisture depletion was not a major factor in determining the moisture stress of this series. At the other extreme, represented by the "0.4 per cent.-salt" series, the osmotic pressure showed such

TABLE IV

THE ESTIMATED LOWER LIMITS OF THE AVERAGE MOISTURE PERCENTAGE AND THE INTEGRATED MOISTURE POTENTIAL FOR THE VARIOUS TREATMENTS

MOISTURE TENSION REGIME	TREATMENT			
	PERCENTAGE ADDED SALT			
	"0"	0.1	0.2	0.4
LOWER LIMITS OF AVERAGE SOIL MOISTURE PERCENTAGE				
	%	%	%	%
Low	14	14	14	14
Medium	10	10	11	11
High	6	7	8	9
INTEGRATED MOISTURE STRESS* BETWEEN THE ABOVE RESPECTIVE LOWER LIMITS AND 20 PER CENT. MOISTURE				
	atmos.	*atmos.*	*atmos.*	*atmos.*
Low	1.1	2.2	4.4	8.2
Medium	1.5	2.8	5.1	9.4
High	3.9	4.6	6.7	10.5

* Arrived at geometrically.

a marked increase with soil moisture depletion that the soil moisture tension was a negligible consideration in the trend of the total moisture stress for this series.

It is difficult to arrive at the integrated moisture stress acting on the absorbing roots between an irrigation and the designated degree of soil moisture depletion. The prescribed degree of water removal from the soil for a given treatment varied from only one day to 10 days (fig. 2). A rough index of the effective moisture stress over an irrigation period may be gained by integrating the respective moisture stress curves within specified limits. These limits of the average soil moisture percentage were available from the data which gave rise to figure 2. The maximum average moisture percentage in each case was 20. The lower limits are given in table IV as well as the integrated moisture stresses. The average green weight per plant is plotted against these respective moisture stresses in figure 9. The closeness with which these paired observations follow a general trend probably involves a modicum of fortuity. It is obvious that these integrated moisture stresses are crude approximations since no information is available as to the status of water relations at the surface of the roots as contrasted with the average

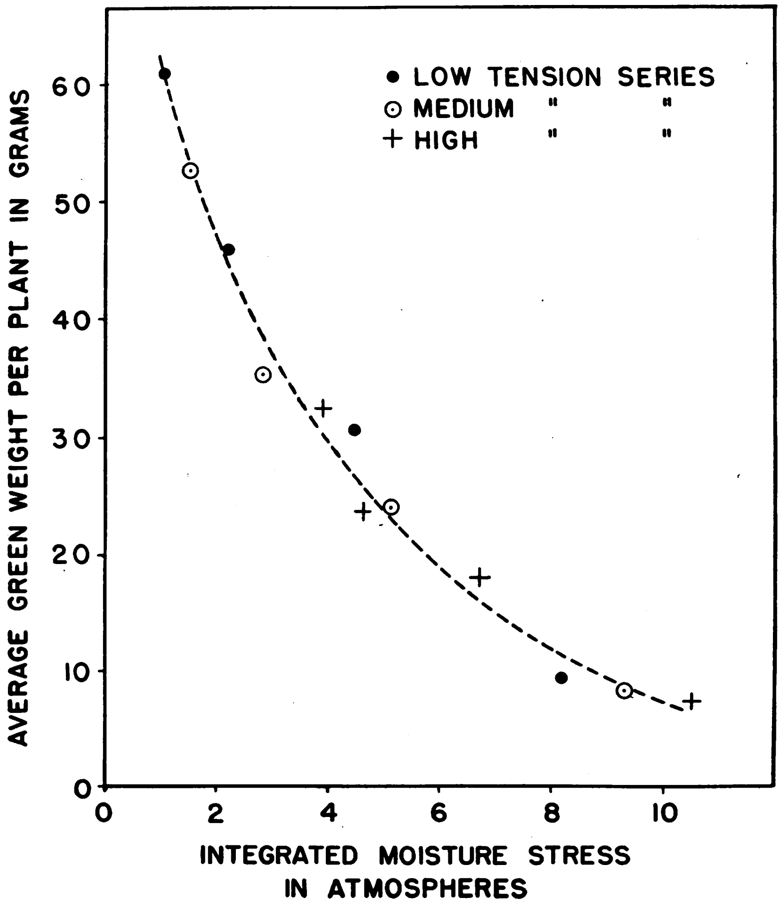

FIG. 9. The relation of plant growth to the "integrated moisture stress."

for the whole soil mass. Nevertheless, a plant has no alternative to precisely integrating all the stresses placed upon it within its inherent limitations. Relative intensities of stress in water supply may be reasonably expected to induce relative inhibitions in growth response. Consequently, the data on growth become approximate evaluations of the relative reliability of the integrated moisture stresses.

The discussion so far has been in support of the view that varying degrees of water availability were a *major factor* in bringing about the observed responses. In continued support of this conclusion, the observations on percentage of starch confirm those of another study (**64**) with regard to the effect of either increasing soil moisture tension or of increasing osmotic pressure of the soil solution in bringing about a depletion in starch reserves within the plant. This may have been due in part to starch hydrolysis through increased amylolytic activity (**52**), but there were no accumulations of the hydrolytic products of starch to compensate for its disappearance. The findings of SCHNEIDER and CHILDERS (**50**), that moisture stress brings about a decrease in apparent photosynthesis and an increase in respiration of apple leaves, are pertinent. The data showed a decreased degree of nitrate reduction under most of the conditions of stress. Nitrogen assimilation is ordinarily a main channel of carbohydrate utilization, and a low level of nitrogen metabolism usually results in carbohydrate accumulation. Since the low rate of growth under conditions of stress would require correspondingly low quantities of synthesized nitrogenous constituents, carbohydrates would accumulate if photosynthetic activity had been maintained at the normal rate. Yet, there was an actual depletion in carbohydrate reserves accompanying intensified soil moisture stresses. It should be noted that during the last few days prior to harvest the temperature of the greenhouse reached a maximum of 93–96° F. and the relative humidity reached a minimum of 25–35 per cent. Such high temperatures are probably superoptimal for photosynthesis in beans. This possibility together with the relatively high rate of transpiration which would be induced by such an aerial environment and which would develop a diffusion pressure deficit within the leaves sufficiently high to inhibit photosynthesis, undoubtedly account for low reserves of photosynthetic products.

The authors believe that factors other than varying degrees of water availability were involved in bringing about differences in the biochemical status of these plants. The trends for reducing sugars in the stems did not appear to be related to moisture tension even though salt concentration in the soil had a marked effect on these sugars. Unpublished observations from investigations reported by MAGISTAD *et al.* (**34**) indicate that bean plants grown on high concentrations of chloride salts tend to develop etiolation in contrast with the deep green color of the leaves of plants grown on cultures with a high concentration of sulphate. In view of this and other studies (**4**, **5**) it appears that an excessive supply of chloride salts is inhibitory to an accumulation of photosynthetic products. Although the indications of such a condition were evident in these plants, it is doubtful if this specific effect of the chloride ion had been a significant factor in the accumulation of dry matter up to the stage of growth considered. In another study (**21**), bean plants also harvested at the stage of incipient flowering showed as great or even greater reductions in growth in the presence of excessive concentrations of sodium sulphate as of sodium chloride at isosmotic pressures.

Some of the foregoing conclusions are not in harmony with observations of other investigators working on allied problems. The fact that an increase in soil moisture tension from 0.2 to 15 atmospheres would necessitate an associated increase in diffusion pressure deficit in the absorbing cells of the roots to initiate the passive entry of water is at variance with the evidence that all soil moisture between field capacity and the wilting percentage [15–18 atmospheres of tension (**13, 44, 53**)] is equally available to plants. Furthermore, some investigators regard the interpretation of the effect of saline substrates on plant growth in terms of osmotic forces as fallacious (**12**). In other words, the effect of the salt in the soil solution is sometimes regarded as chemical (toxicity) rather than physical. From this standpoint, the effect of depletion of soil moisture on increasing the salt concentration of the soil solution in the "0" salt series (table III) suggests the possibility that salt toxicity rather than the increase in soil moisture tension was the active factor in depressing growth in this series. Certainly, the increase in osmotic pressure associated with decrease in soil moisture cannot be disregarded whatever the basis for interpretation. It is evident that if soil moisture is equally available to plants over the moisture tension range of 0–15 atmospheres, then water should also be equally available to plants even though the osmotic pressure of the culture solution was increased from 0.5 to 15 atmospheres by a non-toxic solute. VEIHMEYER and HENDRICKSON (**60**) support this contention with the observation that sunflower plants grown in sand cultures and transferred to sucrose solutions were able to withstand concentrations corresponding to 16 and 20 atmospheres before wilting. Neutral salts affect water absorption and plant growth when the osmotic pressure is only 2 to 4 atmospheres (**11, 22, 23, 29, 34, 37, 48**). However, results of other investigators are not in agreement with those of VEIHMEYER and HENDRICKSON (**60**) as to the magnitude of osmotic pressure of a sucrose solution that plants will withstand without an appreciable effect on water absorption. URSPRUNG and BLUM (**56**), ROSENE (**48**), and LONG (**29**) observed a high degree of inhibition in water absorption by roots when the concentration of sucrose in the substrate was only 4–5 atmospheres. The two latter investigators noted the same effect on water absorption whether the osmotic pressure of the substrate was built up with sucrose or a neutral salt (KNO_3 or NaCl). The preponderance of data indicates that neutral solutes affect water availability to plants by means of osmotic forces even when the osmotic pressure is only a few atmospheres.

The results of some investigators do not support the view that all soil moisture above the wilting percentage is equally available to plants. KRAMER (**28**) found that exudation from the stumps of several species of plants in several different soils ceased when about 45 per cent. of the moisture available to the intact plants was still present. The results suggested that the soil moisture content limiting exudation is as characteristic of a soil as are the wilting percentage and the moisture equivalent, and that active absorption does not take place against a diffusion pressure deficit greater than one or two atmospheres. KRAMER concluded that his findings did not support the theory of equality in availability of soil moisture present in excess of the wilting percentage. Numerous other investigators have found modifications in plant response due to decreasing soil moisture content even though the plants did not wilt (**9, 15, 18, 35, 50, 64**).

The hyperbolic nature of the moisture sorption curve given in figure 1 provides information relevant to the finding that for all practical purposes variations in soil moisture content above the wilting percentage are frequently found to be without influence on plant growth. The general trend of this curve is typical (**61, 62**), but its exact locus will shift with different soils. The nature of this curve shows that most of the available water in the soil is removed even when only one atmosphere of tension has developed. Removal of a small quantity of available water just above the wilting percentage involves an enormous change in moisture tension. Such a rapid rate of change in tension with moisture percentage makes it practically impossible to evaluate a given soil moisture tension of 3 to 15 atmospheres in terms of plant growth. The effective tension at the absorbing surface of the roots is at a given value in this range for only a brief interval, and it is impossible to maintain a given tension in this range (**25, 44**). Consequently, rate of movement of water through the soil to the root membrane probably becomes more of a limiting factor in moisture availability than average moisture percentage in the root zone (**2**).

Moore (**36**) has shown that unsaturated flow of moisture in soils is very low at and below the moisture equivalent. This means that there may be a high gradient in moisture stress between the absorbing surface of the root and the soil particles a few millimeters away. Veihmeyer and his collaborators (*loc. cit.*) have stressed that the range of "available" moisture is meaningless if the roots have not thoroughly permeated the soil mass. In this connection, it is noteworthy that the moisture tension at the wilting percentage may vary from 10 to 24 atmospheres (**6, 60**) or even more (**46**). It is suggested that sunflower plants do not show such an extreme range in their critical diffusion pressure deficit, but that this wide range in the free energy of the soil moisture at wilting percentage reflects inherent inaccuracies in the method. Moreover, it has been shown (**18, 19, 59**) that the wilting percentage is actually a narrow range in soil moisture percentage through which the plant shows increasingly severe wilting. The moisture sorption curve in figure 1 shows why even a narrow range in soil moisture percentage would be associated with a wide range in moisture tension near the wilting percentage.

Summary

Dwarf Red Kidney beans were grown to incipient flowering in 10-gallon containers filled with a loam. These soils contained 0, 0.1, 0.2 and 0.4 per cent. of added sodium chloride on the dry soil basis. The 36 cultures were divided into three moisture tension series. Water was added when the soil moisture tension at the 4-inch depth had reached 250 cm. of water and 750 cm. of water for the first two series, respectively. Water was added to the third series when plants were wilted by mid-morning, corresponding to tension greatly exceeding 800 cm. of water.

Plant growth was inhibited as the soil moisture tension at time of irrigation increased, even though in some of the treatments the soil moisture was always above the wilting range.

Progressive additions of sodium chloride to the soil caused progressive decreases in growth and yield of beans.

Increasing soil moisture tension or salt concentration tended to cause an increase in the percentage of nitrate nitrogen in the plants, and to have a similar effect, though less pronounced, on the percentage of soluble organic nitrogen. Consequently, increasing intensity of either type of stress tended to cause an increase in nitrate/soluble-organic-N ratio. The percentage of protein in the leaves increased with increased intensity of either type of stress.

Soil moisture tension had no effect on percentage of reducing sugars whereas increasing salt concentration was associated with a definite decrease in percentage of these sugars in the stems. The experimental treatments had little effect on percentage of nonreducing sugars or percentage of hemi-reserves. Increasing either soil moisture tension or salt concentration caused a very marked decrease in percentage of starch in the leaves.

The relative effects of salt concentration and soil moisture tension on water availability to these plants is discussed. It is concluded that growth reductions were to a large extent brought about by reduced hydration of protoplasmic proteins, whether water stress was due to osmotic forces or to moisture tension, since adequate percentages of proteinaceous constituents were present for higher rates of meristematic activity.

It is suggested that factors other than varying degrees of water availability were operative in conditioning these plants. The data indicate that excessive concentrations of the chloride ion *per se* affect carbohydrate metabolism, possibly through reduced photosynthetic activity.

It is concluded that the hyperbolic nature of the relationship between soil moisture percentage and moisture tension accounts for the frequent finding that for all practical purposes plants may not show changes in growth response while reducing the moisture percentage of soil from field capacity to nearly the wilting percentage.

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