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EFFECT OF SALINE SUBSTRATE ON HOURLY LEVELS OF CARBOHYDRATES AND INORGANIC CONSTITUENTS OF BARLEY PLANTS

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(WITH SIX FIGURES)

Introduction

Salinity considerations are likely to be an important feature of irrigation agriculture wherever it is practiced. The fact that a depressed growth of many plants results from even moderate accumulations of chloride and sulphate salts in soil solutions has a highly practical significance. From the standpoint of the investigator, it is particularly desirable that the mechanism of salt injury be as clearly understood as our present-day insight into biological processes will permit.

The investigation reported in this paper had the two-fold objective of determining (a) if any relationship existed between salt accumulation in the barley plant and the hourly march or levels of carbohydrate accumulation; and (b) the relation between cyclic changes in transpiration rates and the accumulation of the chemical constituents of the substrate in the shoots of barley plants.

Measurements were made of the concentrations of sugars, starch, and inorganic constituents in 5-week-old barley plants at 4-hour intervals over a 24-hour cycle. The three sets of plants compared were grown in sand cultures supplied with solutions as follows; (a) base nutrient only; (b) base nutrient plus 100 milliequivalents per liter of added chloride; and (c) base nutrient plus 200 milliequivalents per liter of added sulphate.

Materials and methods

Barley was used as the experimental plant; seed of the Sacramento variety was supplied by the University of California. The plants were grown at Riverside, California, in the large, out-of-door, automatically-operated sand culture equipment described by EATON (7). The seeds were planted on April 2,1940, at a depth of one inch, spaced one inch apart in the 30-inch rows, and thinned on April 23 to fifteen plants per row. The sixteen rows of barley per bed were spaced 10.5 inches apart with end rows set 5.5 inches from the ends of the beds.

The composition of the three culture solutions is shown in table I.

The nutrient solutions were automatically pumped onto the beds twice

1 Contribution no. 20 of the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Riverside, California, in cooperation with the Western States.

TABLE I

COMPOSITION OF CULTURE SOLUTIONS*

SOLUTION	MILLIEQUIVALENTS PER LITER								PARTS PER MILLION			CONDUCTANCE $K \times 10^5$ AT 25° C.	OSMOTIC CONCENTRATION
	Ca	Mg	Na	K	Cl	SO_4	PO_4	NO_3	Cl	SO_4	TOTAL IONS		
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*atm.*
Base nutrient	6.2	5.4	1.9	3.2	4.0	4.6	0.6	8.0	.....	.....	1,071	178	0.736
100 m.e./L. of Cl	26.2	35.4	41.9	3.2	104.0	4.6	0.6	8.0	3,550	.....	6,532	1,172	4.192
200 m.e./L. of SO_4	26.2	85.4	101.9	3.2	4.0	204.6	0.6	8.0	.....	9,600	14,361	1,388	4.158

* Iron was supplied by 0.2 per cent. of magnetite mixed with the sand. Trace elements were supplied to give: boron, 0.6 p.p.m.; Mn, 0.2 p.p.m.; and, from impurities in the salts, approximately 0.2 p.p.m. of zinc.

daily until May 6, after which time the flushing hours were 7 : 00 A.M., 9 : 00 A.M., and each hour thereafter until and including 6 : 00 P.M. Each culture solution was maintained at pH 6.0 by the addition of small amounts of concentrated nitric acid (8).

All cultures were supplied with base nutrient only from April 2 to April 5, 1940. On April 5 the salt beds received one-fifth of the amount of salt that was to be added to bring them to the above-stated concentrations. The second, third, fourth, and fifth increments of salt were added to the salt beds on the 11th 12th, 14th, and 17th of April, respectively.

The plants, tops only, were harvested over a 24-hour period. Two rows of plants were cropped from each bed every four hours beginning at 4: 00 A.M. on May 10th, and ending at 4: 00 A.M. on May 11th, 1940. The second and ninth rows were removed first, the third and tenth at 8 A.M., continuing on in sequence until all but the first and sixteenth rows (outside rows) had been taken out. These latter were removed on the third morning and used as preliminary test material in the analyses.

Each of the rows was harvested, weighed, and analyzed separately. After a fine chopping of each row, a lo-gram (fresh weight) sample was put up in boiling alcohol for sugar analyses. The remainder of each row sample was dried to determine the percentage of moisture, and also to furnish the dry matter on which the inorganic analysis and starch were to be determined.

The designations "reducing sugars," "sucrose," and "starch" are used in the conventional and well-recognized manner without assuming that the entire amount of the reducing materials measured were these specific substances. Technique and reagents were carefully standardized to insure a high degree of dependability in comparisons between treatments and hours of harvest.

SUGARS

REDUCING SUGARS.–The finely-chopped, 10-gram samples of plant material were dropped into boiling ethyl alcohol of such strength that the final concentration would be approximately 80 per cent. and the boiling continued for approximately five minutes to insure complete penetration of the alcohol and the cessation of protoplasmic and enzymatic activity.

For extraction, 125 ml. of 80 per cent. ethyl alcohol was added to the sample and the whole placed in a Soxhlet extractor of the newer type in which the extracting alcohol in the thimble approaches the temperature of the liquid in the receiving flask.

The alcohol was evaporated from the extract on a steam bath, the resulting aqueous extract cleared with neutral lead acetate, and deleaded with disodium hydrogen phosphate according to HASSID'S method (12). The mixture was then made to 100 ml. and filtered through a dry filter discard-

ing the first 2 or 3 ml. of filtrate. Five-ml. aliquots were used for the determination of reducing sugars following a modified HARDING and DOWNS method described by VAN DER PLANK (18).

TOTAL SUGARS.-The pH of a 50-ml. sample of the foregoing solution was adjusted to 4.9 with a few drops of 10 per cent. acetic acid, 2 to 4 drops of a 1 per cent. solution of WALLERSTEIN invertase scales added, and the solution then hydrolyzed overnight at room temperature (12). The following morning the solution was made to 100 ml. and filtered through a dry filter discarding the first 2 or 3 ml. of filtrate. Five-ml. aliquots were used as before for the determination of reducing substances.

SUCROSE.–This value was obtained by subtracting the reducing-sugar value from the total-sugar value.

STARCH

Fresh samples of the plant material were dried and ground, then redried in an oven at 110" C. for 2 hours, and allowed to cool in a desiccator before weighing. When possible, a 5-gram sample was used for analysis. The procedure of HASSID, *et al.* (13) was followed for the solubilization and extraction of starch. The extract was hydrolyzed overnight with pancreatin-free plant diastase at room temperature. After hydrolysis the solution was cleared according to the method described by HASSID (12), and reducing sugars again determined as before.

INORGANIC ANALYSES

The official methods of the Association of Official Agricultural Chemists (1) for plant materials were followed for the determination of calcium, magnesium, total sulphur, total phosphorus, and total nitrogen. Sodium was determined by the uranyl zinc acetate method, and potassium by a cobaltinitrite procedure.

Weather conditions

Overcast skies and relatively cool weather predominated during the period preceding the day of sample collection. Between April 16 and May 9 the average maximal temperature was 76.8" F. and the average minimal, 49.3" F. The average relative humidity at noon was 41 per cent. and average wind movement was 43.1 miles per 24 hours.

On the day of harvest, May 10, 1940, the sky was clear at 8 : 00 A.M., partially cloudy at noon and also at 4: 00 P.M. On May 11, the sky was clear by 8: 00 A.M. The air temperatures, at 4-hour intervals from 4: 00 A.M. Friday, May 10th, to 4: 00 A.M., Saturday, May llth, were as follows: 57.5, 75.0, 98.0, 91.0, 72.0, 62.0, and 57.0" F., respectively. The relative humidities for the same hours were: 62, 32, 13, 35, 56, 86, and 82 per cent., respec-

tively. Evaporation from a black shallow pan (water 1 cm. in depth and 1,000 sq. cm. in area,) evaporimeter was as follows : 4-8 A.M., 79 gm. ; 8 A.M.-noon, 412 gm. ; noon--4 P.M., 547 gm. ; 4 P.M.--8 P.M., 149 gm. ; 8 P.M.--midnight, 8 gm. ; and, midnight to 4 A.M., 6 gm.

Results

GROWTH

A close-up view of the barley plants preceding harvest is shown in figure 1.

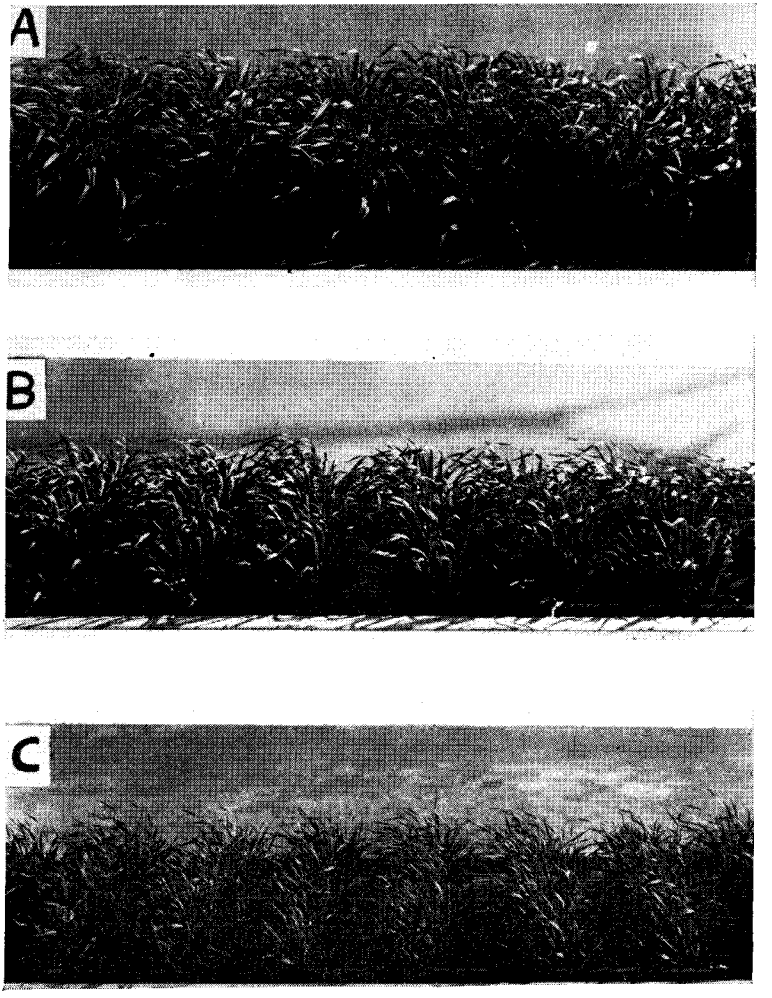

FIG. 1. Close-up view of barley plants on day preeeeding harvest. A. Control. B. 100 me. of chloride per liter. C. 200 me. of sulphate per liter.

The march of dry weight of tops (based on 30-plant samples) through the 24-hour cycle is shown for each of the three treatments in figure 2, and the data are summarized in table II. By analysis of variance there is a highly significant difference between the weights of the plants in each of the three treatments. Growth was depressed more by 200 m.e. per liter of sulphate than by 100 m.e. per liter of chloride, which is in accord with the toxicity of the two ions found in earlier investigations with barley (9, table III).

TABLE II

EFFECT OF TRESTNENT ON DRY WEIGHT AND MOISTURE CONTENT OF BARLEY SHOOTS

TREATMENT	FRESH WEIGHT	DRY WEIGHT	MOISTURE	ON RELATIVE BASIS		PERCENTAGE REDUCTION IN WEIGHT	
				FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT BASIS	DRY WEIGHT BASIS
	gm.	gm.	%			%	%
Control	6,396	598	90.6	100	100	0.0	0.0
100-Cl	2,778	367	86.8	43	61	57.0	39.0
200–SO_4	1,817	259	85.7	28	43	72.0	57.0

The slopes of the curves in figure 2 show an hour-to-hour increase in the weight of the control barley shoots throughout the 24-hour cycle, but little or no increase is shown in the weight of the chloride or sulphate shoots after noon. This finding could mean either that carbohydrates were only slowly synthesized in the salt plants after noon or that most of the photosynthetic product was translocated to the roots.

The continued weight increase of the control plants throughout the day indicates a more rapid synthesis of carbohydrates in these than in the salt plants. The lower sugar (fig. 5) and starch concentrations (fig. 6) in the control than in the salt shoots indicates, furthermore, a more rapid conversion of the photosynthetic product of the control plants into tissue structures than occurred in the salt plants. A greater accumulation of carbohydrates in the *roots, as* well as in the shoots, of the salt plants is implied by the fact that the control barley plants cropped at 4 A.M., May 10, died without developing new shoots from the stubble, whereas new shoots were developed from the stubble of all other croppings (see in this connection the carbohydrate data of figs. 5 and 6). The fact that shoots did develop from the stubble of the control plants cropped at 4 A.M. on the second morning (May 11) indicates that the slightly higher concentration of carbohydrates found in these plants on the second morning was above the minimum required for continued growth of shoots. Evidence of a higher proportionate translocation of carbohydrates to the roots of cereals grown on saline

than on non-saline substrates has been provided elsewhere (9) by the finding that the weight of roots of wheat in high-chloride solutions and corn in high-chloride and high-sulphate solutions was markedly greater than that found for corresponding control plants.

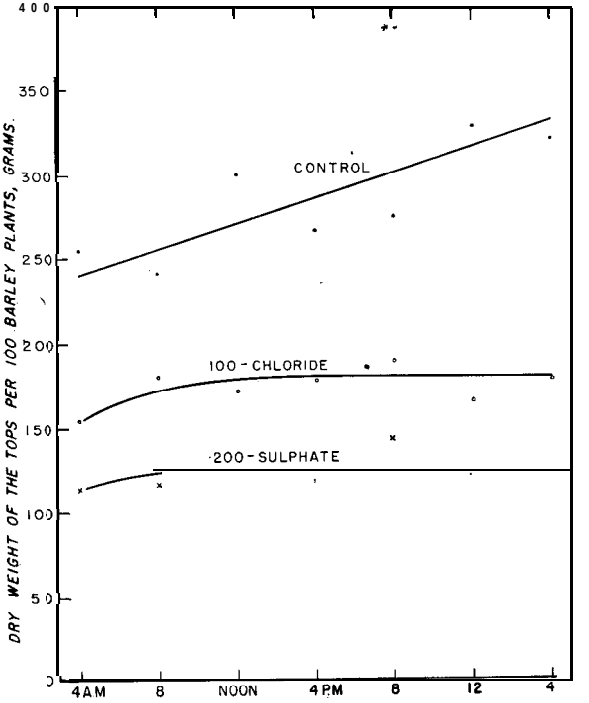

FIG. 2. Dry weight of tops per 100 barley plants at successive sampling hours.

MOISTURE CONTENT

The barley plants on the chloride substrate maintained a significantly lower average moisture content in their shoots than did the control plants and the sulphate plants operated at a significantly lower moisture level than the chloride plants (fig. 3). The differences found during the night hours, when evaporation was negligible, were only slightly more marked during the late afternoon when evaporation was high. There was thus a basic change in the tissue hydration characteristics of barley grown on saline substrates that is not related to evaporation rates. In another work (9), the succulence of barley as measured by the amount of sap that could be expressed from frozen tissue under uniform pressures was found to decrease sharply as the amount of either chloride or sulphate salt in the substrate was increased. No such effect was shown by milo, alfalfa, cotton, tomato, or sugar beet. In unpublished results SOKOLOFF and EATON found that dried and ground bar-

ley shoots grown on a sodium chloride substrate swelled and formed a nearly-impermeable mass when leached with water on a BUCHNER funnel, whereas control and high-calcium barley plant material was easily extracted.

The decreasing moisture percentages through the three sets of plants (control-chloride-sulphate) are associated (fig. 4) with decreasing con-

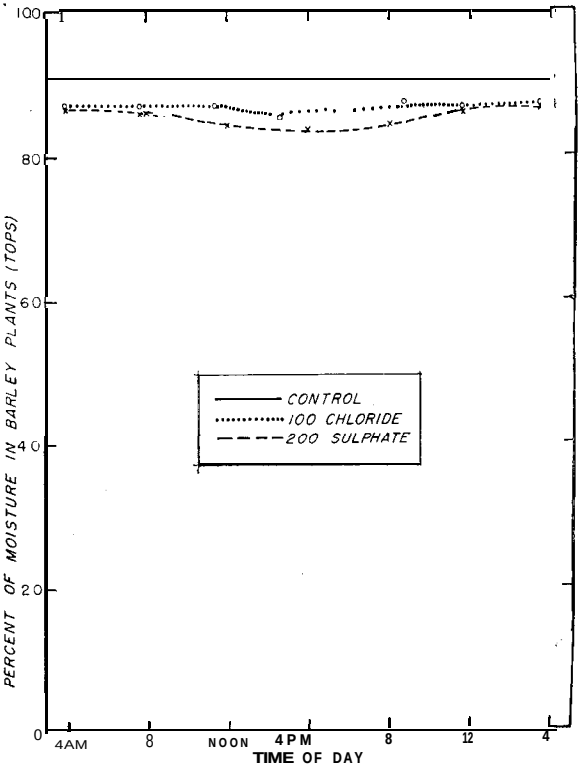

FIG. 3. Percentage of moisture in the tops of barley plants as related to treatment and time of day.

centrations of calcium, unchanged magnesium, sharply increasing sodium, decreasing potassium, and higher sucrose and starch concentrations in the shoots. From such diverse associations it would be difficult to assign a causal relationship to any one constituent. This is particularly true when it is recalled that GREGORY and SEN (11) reported "progressive potassium starvation leads to continuous increase in water content (in barley), while nitrogen deficiency has the reverse effect."

SALT ACCUMULATION

Little published evidence exists on the relationship between salt accumulation in plants and transpiration rates and yet in terms of salt toxicity and

the broad differences in climatic conditions found throughout irrigated regions this question can be regarded as basic. MUENSCHER (16), in general agreement with the findings of numerous earlier investigators who in nearly all instances confined their laboratory measurements to the determination of total ash, found that the ash of barley expressed in percentage of total dry weight of the entire plants varied but slightly regardless of whether the plants were grown under conditions of high or low transpiration and irre-

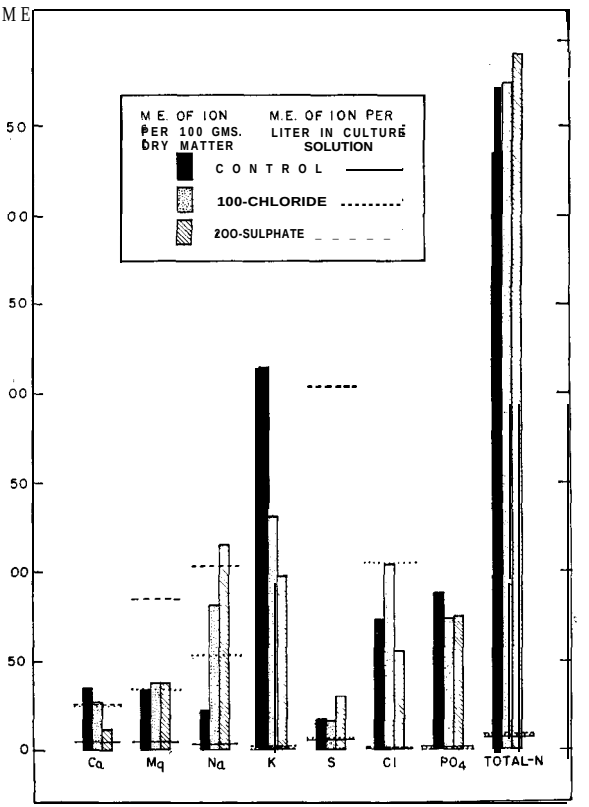

FIG. 4. The chemical composition of 5-week-old barley plants grown in base nutrient, in high chloride, and in high sulphate solutions.

spective of how transpiration was reduced. HOAGLAND and BROYER[2] on the other hand have found higher concentrations of sodium and chloride ions in the expressed sap of barley plants grown in a dry chamber than in that of plants grown in a humid chamber. They also report that the bromide ion is taken up from culture solutions and moved into roots, stems, and leaves

[2] HOAGLAND, D. R., and BROYER, T. C. Unpublished data presented at the 22nd annual meeting of the Pacific Division of the A.A.A.S., San Diego, California, June 21, 1938.

of squash and cotton more rapidly under the influence of light and humidity conditions conducive to high transpiration rates than under those conducive to low transpiration rates. Respiration rates and the availability of carbohydrates (14) as well as the oxygen supply to inner and outer root tissues (5) are now indicated as being factors that may markedly affect salt accumulation in the shoots of plants and are accordingly relevant considerations.

It seemed probable in advance of this study that if major relations existed between cyclic environmental factors and salt accumulation, the effect would be evident in comparisons of the concentrations found in this remarkably uniform series of plants as sampled in the late afternoon under conditions of high transpiration rates and at night when transpiration was negligible. In this connection it is desirable to recall that on the day covered by the samples the temperature rose to nearly 100" F. and the relative humidity dropped to 13 per cent.

FONDER (10) has reported on the variations in the amounts of calcium and magnesium of the alfalfa plant at different hours of the day. The plants were grown on Hillsdale sandy loam, and they were sampled at 4 A.M., 8 A.M., noon, 4 P.M., 8 P.M., and midnight. The leaves and stems were analyzed separately for percentage of moisture, calcium, and magnesium. He reported variations with time of day in the calcium, magnesium, and moisture contents in the stems and leaves, and variations in the calcium and magnesium contents of the expressed sap.

In this study separate analyses were made of each harvest of barley (as cropped in duplicate at succeeding 4-hour periods over the 24-hour cycle) for the following elements: Ca, Mg, Na, K, S, Cl, PO,, and total nitrogen. As shown in table III, wherein the individual analyses are all reported, no evidence indicates that the extreme variation in transpiration rates over the 24-hour cycle has any effect on the accumulation of any ion in the tops of these barley plants. Furthermore, the period of most rapid carbohydrate accumulation coincided in part with the most rapid influx of water. Over this period, as pointed out by CANNON (2), the oxygen supply both from the substrate and from photosynthesis should have been at a maximum, yet there is no evidence that salt accumulation was thereby augmented. Accordingly, the conclusion would follow that neither oxygen nor labile respiratory materials fell at any time below the concentrations required for maximum salt absorption and retention in the shoot. Under the conditions of this experiment, the accumulation of inorganic constituents was affected only by the concentrations of the substrate. The extent of accumulation of a particular ion (fig. 2) is not dependent alone upon its concentration in the substrate; but instead, as has been extensively observed by other investigators, is dependent as well upon the uptake of other ions and their effects on the plant.

TABLE III

INORGANIC COMPOSITION OF 5-WEEK-OLD BARLEY PLANTS AT FOUR-HOUR INTERVALS IN A 24-HOUR CYCLE, AND THE COMPOSITION OF THE SUBSTRATE SOLUTIONS

TREATMENT: HOUR OF DAY	MILLIEQUIVALENTS PER 100 GRAMS OF DRY MATTER							
	Ca	Mg	Na	K	S*	Cl	PO_4	TOTAL NITROGEN*
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Control plants								
4:00 A.M.	39 35	41 36	22 19	226 235	21 21	78 72	88 92	354 311
8:00 A.M.	36 36	36 36	22 19	218 216	19 13	66 63	88 83	358 352
Noon	34 31	31 31	26 20	238 216	19 13	71 66	83 80	339 355
4:00 P.M.	34 36	34 36	17 24	201 253	17 15	65 82	90 87	316 324
8:00 P.M.	37 39	36 38	23 24	239 254	21 18	76 80	88 78	323 319
Midnight	30 35	34 38	24 21	221 233	15 16	75 76	88 86	321 352
4:00 A.M.	30 34	32 37	18 24	220 223	15 15	66 73	89 85	311 360
Average	35	35	22	228	17	72	86	335
100-chloride plants								
4:00 A.M.	29 28	42 40	74 85	126 132	16 17	108 105	77 73	382 394
8:00 A.M.	26 27	36 39	77 77	133 128	17 18	103 101	75 71	385 368
Noon	26 26	37 39	77 80	128 132	15 14	104 109	77 72	388 380
4:00 P.M.	26 25	38 38	74 74	129 130	16 13	99 101	73 69	377 372
8:00 P.M.	27 27	38 32	82 84	136 140	13 18	106 107	90 67	363 347
Midnight	25 26	39 39	86 84	119 132	17 16	93 106	75 63	375 370
4:00 A.M.	26 26	39 40	85 85	126 118	17 16	91 92	79 70	373 383
Average	26	38	80	129	16	102	74	376
200-sulphate plants								
4:00 A.M.	12 12	37 37	122 121	127 92	31 27	54 55	55 72	399 375
8:00 A.M.	10 12	36 36	114 119	90 99	26 26	51 54	76 82	409 370
Noon	11 11	28 36	110 115	94 84	25 30	59 52	74 76	389 413
4:00 P.M.	11 10	37 30	115 105	91 98	26 27	51 58	79 73	395 380
8:00 P.M.	10 10	43 37	110 111	97 93	26 26	56 56	66 82	371 380
Midnight	11 12	40 38	116 125	93 97	31 27	53 55	78 81	376 399
4:00 A.M.	12 11	41 40	118 120	99 95	27 33	54 52	79 81	406 403
Average	11	37	116	96	28	54	75	390

* Total nitrogen and sulphur in the plant tissue reported on the basis of 0.014 grams N and 0.016 grams S per milliequivalent.

Calcium accumulation in the barley shoots was depressed by the addition of 20 m.e./l. of calcium to the culture solution when accompanied by the addition of other salts in amounts sufficient to raise either the chloride level of the culture solution from 4 to 104 m.e./l. or the sulphate level from 4.6 to 204.6 m.e. per liter. The magnesium level in the plants was unaffected by raising the concentration of magnesium in the substrate from 5.4 to 35.4 m.e./l. in the chloride solution and to 85.4 m.e./l. in the sulphate solution.

The sodium levels in the barley shoots were more directly related to the concentration of sodium in the culture solutions, than were the levels of any other ion. The control solution contained 1.9 m.e./l. of sodium and the plants contained 21.6 m.e. per 100 grams of dry material. An increase of 50 m.e./l. of sodium added as sodium chloride to the culture solution resulted in an increase of 59 m.e. per 100 grams of dry matter in the shoot; the addition of 100 m.e./l. of sodium sulphate to the base nutrient raised the level of sodium in the plant 96 m.e. per 100 grams of dry matter.

With no change in the potassium level in the culture solutions the addition of calcium, magnesium, and sodium salts to the solution in amounts sufficient to raise chloride by 100 m.e./l. resulted in decreased potassium in the plant from 228 m.e. to 129 m.e. per 100 grams of dry matter. With the addition of 200 m.e./l. of sulphate salts to the base nutrient the potassium level in the plant dropped to 96 m.e. per 100 grams of dry matter. The loss in potassium in the plant material may be related to sodium uptake, in the case of barley, but it is to be noted that the loss in potassium is not proportional, in the three sets of plants, to the gain in sodium.

Adding 200 m.e./l. of sulphate ion to the nutrient solution increased the sulphate level in the plant from 17.0 to 27.5 m.e. per 100 grams of dry matter.

With 4 m.e./l. of chloride in the culture solution the control plants accumulated 72 m.e. per 100 grams of dry matter. Adding 100 m.e./l. of chloride to the nutrient solution increased the chloride in the plant to 102 m.e. per 100 grams of dry matter. The addition of sulphate to the nutrient solution reduced chloride accumulation substantially but the addition of chloride was without effect on sulphate accumulation.

Nitrogen and phosphate accumulation was little affected by the addition of the chloride and sulphate salts to the base nutrient, notwithstanding marked changes in the concentration of other ions in the plant. The presence of the slightly larger quantities of nitrogen in the chloride and sulphate plants than in the control plants may indicate accumulation of unutilized nitrate ion associated with depressed growth but the minor reduction in phosphate with the salt additions remains unaccounted for.

In an earlier study (9) that involved analyses of the expressed saps of milo, alfalfa, cotton, tomato, and sugar beet grown together on substrates

with added chloride and sulphate salts, the accumulation responses of the different plants were found to be diverse. The present data on barley serve to emphasize further that great specificity exists among the plants in their accumulation characteristics and inter-ionic effects. These results from barley plants are especially noteworthy in the fact that sodium accumulation apparently depressed potassium accumulation but as is characteristic of many plants the accumulation ratio of potassium in the plant to that in the substrate is far higher than the sodium ratio. Barley, by these data, accumulates substantially more sodium than many other plants that have been studied (4, 9, 19). The 50-fold increase in the sodium concentration in the sulphate substrate brought about only a 5-fold increase in the sodium concentration in the plant.

DELEANO and GOTTERBARN (6) working with barley concluded that there is "antagonism" among the elements, calcium, magnesium, and potassium.

CARBOHYDRATES

The cyclic accumulation of carbohydrates in plant tissues has been extensively investigated but only a few citations will be made to this literature for the reason that in the present work the point of primary concern is that having to do with the effect of saline substrates on carbohydrate accumulation.

SUGARS.-PUHR and HUME (17) found that the maximal amount of sucrose and total sugar in the leaves of corn occurred between 1 and 4 P.M. MILLER (15) working with the leaves of corn and sorghums obtained maxima varying between noon and 5 P.M. CLEMENTS (3) observed maximal total sugar concentrations in the leaves of sunflower, potato, and soybean varying from 2 to 5 P.M. In the present experiment, for all treatments, the maximal concentration of sucrose and total sugar occurred in the samples taken at 4 P.M.

As shown in figure 5 and in table IV, the concentration of reducing sugars in fresh barley shoots was not materially affected by a differential accumulation of calcium, sodium, or potassium in the plant tissue nor by differences in the chloride and sulphate concentrations of the plant material. MILLER (15), whose findings are in accord with those of PUHR and HUME (17) on corn, reported that in corn and sorghum leaves the reducing sugars, as a rule, showed very little increase during the day and that the amount present at the different periods of the day was very irregular.

The concentration of the sucrose fraction was markedly affected by the salt treatments. Sucrose was substantially higher at nearly all hours of the day in the chloride plants than in the control plants and again higher in the sulphate plants than in the chloride plants.

The average concentrations (milligrams of sugar per IO-gram fresh plant

material) of total sugar in the three sets of plants (control, chloride, and sulphate) over the 24-hour period were 44.4, 58.4, and 67.9 mg., respectively. The 4 P.M. (maximal) values were 65.1, 97.2, and 106.6 mg., respectively.

The accumulation of sugars in the fresh tissue of the barley shoots in the three treatments stands in the reverse order of growth. It is thus fully evident, in the case of barley, that salt accumulation does not reduce the concentration of sugars and, in turn, that reductions in available sugars are

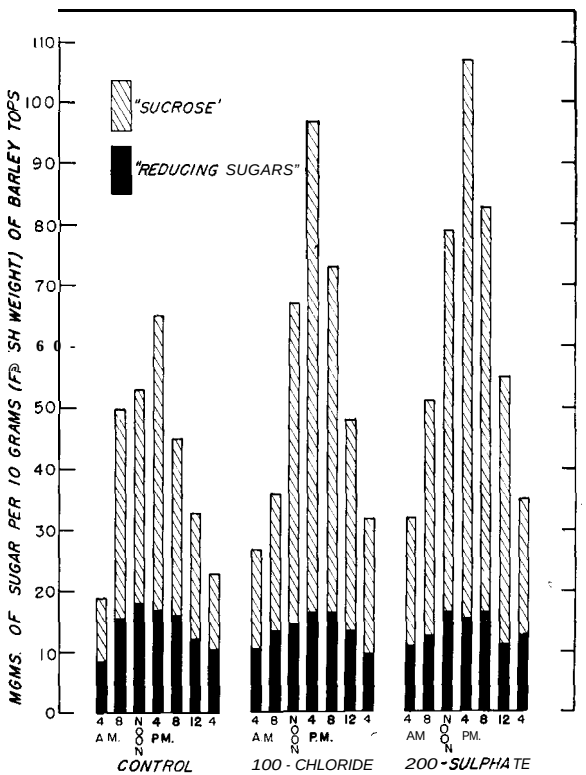

FIG. 5. The concentration of sugars in the shoots of 5-week-old barley plants, as affected by treatment and time of day.

not a direct or intermediate causal factor that will account for the reduction in growth on the saline substrates. The failure of the chloride and sulphate plants to utilize accumulated sugar and starch (see next section) in cell elaboration as rapidly as the control plants cannot be attributed to nitrogen or phosphorus deficiency since the levels of these elements differed little in the three sets of plants. The possibility remains that the depression in potassium concentration by the salt additions was responsible for curtailed

growth, but against this hypothesis is the fact that it would be difficult to argue insufficient potassium in plants containing 96 or 129 m.e. per 100 grams of dry matter. Furthermore, it has been shown elsewhere (9) that potassium depression does not occur in all plants with sodium accumulation. The fact of depressed growth and carbohydrate accumulation might on the barley evidence be attributed directly to sodium; but again, sodium accumulation does not always occur. Milo, for example, accumulates only a negligible amount of sodium and yet it is injured by salt concentrations such as those here used (9). The writers would hesitate to argue that the mechanism of salt injury is identical in different species; for it is highly hazardous to expound a general explanation on the basis of biochemical changes found to take place in some one plant.

TABLE IV

MILLIGRAMS OF SUGAR PER 10 GRAMS (FRESH WEIGHT) OF 5-WEEK-OLD BARLEY SHOOTS

TREATMENT: HOUR OF DAY	SUCROSE			REDUCING SUGARS			TOTAL SUGARS		
	EAST* ROW	WEST ROW	AVERAGE	EAST ROW	WEST ROW	AVERAGE	EAST ROW	WEST ROW	AVERAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Control:									
4 A.M.	10.3	11.1	10.7	8.7	8.5	8.6	19.0	19.6	19.3
8 A.M.	27.1	41.3	34.2	14.5	16.7	15.6	41.6	58.0	49.8
Noon	27.6	42.3	34.9	18.0	17.7	17.9	45.6	60.0	52.8
4 P.M.	56.0	40.5	48.2	16.0	17.7	16.9	72.0	58.2	65.1
8 P.M.	33.4	23.7	28.5	16.6	15.7	16.2	50.0	39.4	44.7
Midnight	17.1	23.5	20.3	13.1	11.5	12.3	30.2	35.0	32.6
4 A.M.	13.2	12.0	12.6	10.2	11.0	10.6	23.4	23.0	23.2
100-chloride									
4 A.M.	16.1	16.9	16.5	9.7	11.5	10.6	25.8	28.4	27.1
8 A.M.	20.4	23.8	22.1	13.0	14.2	13.6	33.4	38.0	35.7
Noon	52.8	52.0	52.4	16.2	13.0	14.6	69.0	65.0	67.0
4 P.M.	82.8	79.2	80.6	17.0	16.0	16.6	99.2	95.2	97.2
8 P.M.	58.6	55.0	56.8	17.0	16.0	16.5	75.6	71.0	73.3
Midnight	33.9	35.6	34.7	12.1	14.6	13.4	46.0	50.2	48.1
4 A.M.	22.8	21.4	22.1	9.0	10.0	9.5	31.8	31.4	31.6
200-sulphate									
4 A.M.	20.2	22.5	21.3	11.0	10.7	10.9	31.2	33.2	32.2
8 A.M.	38.3	37.7	38.0	11.7	13.3	12.5	50.0	51.0	50.5
Noon	56.0	69.5	62.6	17.0	15.5	16.4	73.0	85.0	79.0
4 P.M.	93.0	89.3	91.1	14.2	16.7	15.5	107.2	106.0	106.6
8 P.M.	71.4	61.4	66.4	14.0	19.8	16.9	85.4	81.2	83.3
Midnight	43.6	42.9	43.2	11.0	11.7	11.4	54.6	54.6	54.6
4 A.M.	24.7	20.4	22.5	12.5	13.0	12.8	37.2	33.4	35.3

* "East" and "west" refer to the duplicate rows that were harvested at each sampling period—one row of which was in the eastern half of the bed, and one row of which was in the western half of the bed.

STARCH.—The effect of substrate on the starch percentage was found to be marked during the late hours of the day and the differences between the

treatments (fig. 6) were statistically significant. The 4 to 8 P.M. maxima for the control, chloride, and sulphate plants were 1.00, 1.30, and 1.55 per cent., respectively.

The fact that the higher starch levels in salt plants are found to be associated with higher sugar levels on the fresh weight basis is regarded as indicating that polymerization is directly related to sugar concentrations in leaf sap.

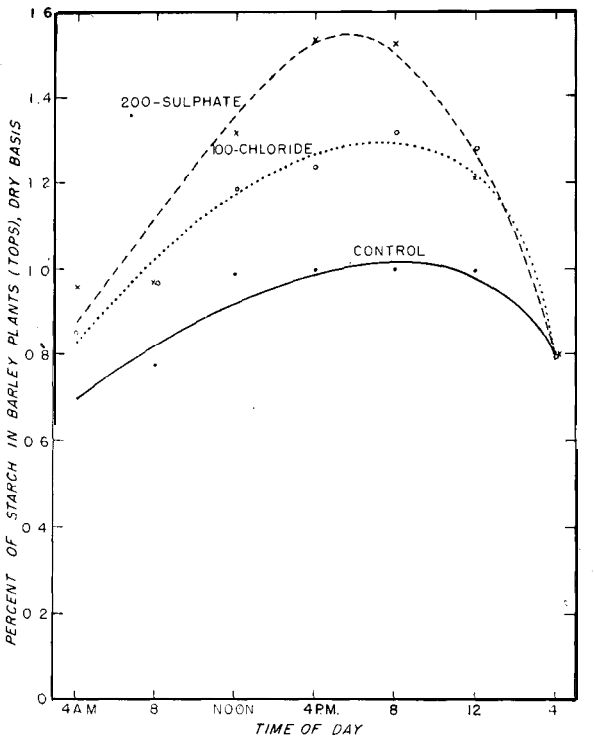

FIG. 6. Percentage of starch (dry weight basis) in the tops of barley plants.

That the enzyme system concerned with starch formation and starch hydrolysis was not impaired or inactivated by salt accumulation is clearly indicated by the rise and fall in starch accumulation. There is no clue in the starch cycle as to a mechanism of salt injury to plants.

Summary

1. With the purpose of determining (a) the relation between cyclic variations in transpiration rates on the accumulation of inorganic constituents in barley and (b) the effect of saline substrates on the accumulation of carbohydrates, barley plants were grown in sand cultures supplied with a base nutrient, base nutrient plus 100 m.e./l. of chloride, and with the base

nutrient plus 200 m.e./l. of sulphate. The plants were sampled at 4-hour intervals over a 24-hour cycle and analyzed for mineral constituents and carbohydrates.

2. The relative weights of the control, 100-chloride, and 200-sulphate plants on a fresh weight basis were : 100, 43, and 28 ; and on the dry weight basis: 100, 61, and 43, respectively. The average moisture content of the three sets of plants was: 90.6, 86.8, and 85.7 per cent., respectively.

3. Under all treatments essentially uniform concentrations of calcium, magnesium, sodium, potassium, sulphate, chloride, phosphorus, and nitrogen in the dry matter were found over the 24-hour cycle in which the temperature rose to 98" F. and the relative humidity dropped to 13 per cent. at noon.

4. The addition of both chloride and sulphate salts (24-hour averages) reduced calcium concentrations relative to the control plants, left magnesium unchanged, caused a marked increase in sodium, a marked decrease in potassium, and had little effect on total nitrogen and phosphate concentrations.

5. The accumulation of chloride in the control plants was notably high (72 m.e. per 100 grams dry matter), and the addition of 100 m.e./l. of chloride to the substrate brought about a further uptake to 102 m.e. per 100 grams of dry matter.

6. The control plants contained 17 m.e. per 100 grams of dry matter of sulphur and the 200-sulphate plants contained 27.7 m.e. per 100 grams of dry matter.

7. Associated with the foregoing increased concentration of sulphur in the plant material (sulphate plants) there was a depression in the amount of chloride (72 to 54 m.e. per 100 grams dry matter), but there was no change in the content of sulphur (chloride plants) associated with chloride accumulation.

8. At 4 P.M. the concentration of carbohydrates in the control, chloride, and sulphate plants were, respectively: reducing sugars-16.9, 16.6, and 15.5; sucrose-48.2, 80.6, and 91.1; total sugars-65.1, 97.2, and 106.6 milligrams per 10 grams fresh weight of tops. In terms of percentage of dry weight, the starch values were, respectively: 1.0, 1.2, and 1.5 per cent.

9. The accumulation of carbohydrates was thus associated with salt accumulation, indicating that the salts interfered with the utilization of carbohydrates in cellular elaboration rather than with photosynthetic activity.

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