Sodium as an Essential Micronutrient Element for a Higher Plant (Atriplex vesicaria)[1]

P. F. Brownell

Department of Botany, University of Adelaide, South Australia

Introduction

This paper presents evidence that sodium is essential for the growth of *Atriplex vesicaria* Heward ex Benth. (Bladder salt bush). Prior to a preliminary report of this work (4), Allen and Arnon (1) had shown sodium to be essential only for the blue-green alga *Anabaena cylindrica*. The optimum growth of the alga was obtained in media containing 218 μeq/liter (5 ppm) or higher of sodium. The requirement for sodium was specific; lithium, potassium, rubidium, and caesium did not substitute for sodium.

The effects of sodium on higher plants have not been clear. In many observations the dry weight production of plants growing in the field or in soil culture has increased following the application of salts of sodium. The literature dealing with such observations has been reviewed by Harmer and Benne (5), Lehr (9,10) and Wybenga (17). The results of these investigations although of possible economic significance give little information on the actual role of sodium in plant nutrition. Such increases in yield could have been due to the effects of the salt in either modifying the soil, or in increasing the uptake of the other ions or to the anion associated with the sodium being involved in the nutrition of the plant.

An increase in dry weight occurs following the application of salts of sodium to various higher plants growing in low potassium culture solutions (13). This would suggest that sodium partially replaces potassium in some species. There are also suggestions in the literature that the dry weight of some plants, mostly members of *Chenopodiaceae*, increases in solutions containing adequate potassium following the application of sodium (6,8,14,16). This latter evidence suggests that sodium may have an independent role as a nutrient element.

The possibility existed that higher plants might require sodium, but in such small amounts that these needs were always satisfied where plants grow under natural conditions. If so, evidence that sodium is essential for higher plants would be obtained by studying their growth only under conditions in which sodium was rigorously excluded. Such conditions, due to the ubiquity of sodium, are difficult to achieve, and the possible sources of sodium, the seed, nutrient salts, water, culture vessels, and the atmosphere, were critically examined.

This paper describes techniques used to reduce the sodium contamination from these sources and a series of experiments in which sodium was shown to be essential for *Atriplex vesicaria*. This species occupies large areas of arid Australia, and was chosen for investigation as it accumulates large quantities of sodium in its leaves (15).

Materials and Methods

Determination of Sodium. Sodium was determined in distilled water by carefully evaporating it down to one-thousandth of its original volume in a silica beaker. A filter flame photometer was used in these determinations.

When estimating sodium with a filter flame photometer in solutions of nutrient salts, particularly in those of calcium and potassium, the relative errors due to interference by other ions increased as the salts were progressively purified. Interference was also experienced to a less extent, in the estimation of sodium in solutions containing iron, boron and phosphate. Estimations of sodium with an atomic absorption instrument (3) were virtually free of interference but when estimating sodium in highly concentrated solutions of nutrient salts and digests of plant material, the response by the instrument to sodium was reduced. Under these conditions, it was necessary to prepare calibration curves showing the responses of the atomic absorption instrument to known concentrations of sodium in solutions similar to those in which sodium was to be estimated.

Sodium was estimated in plant material by the following method. From finely ground plant material 2 representative fractions were taken of less than 0.5 g. These were dried at 95°, until they had reached constant weight, and then placed in a desiccator. When at room temperature, they were reweighed and placed in quartz Kjeldahl tubes of approximately 15 ml capacity and digested with 1 ml H_2SO_4 (S.G. 1.86), 1 ml $HCLO_4$ (S.G. 1.70), and 5 ml HNO_3 (S.G. 1.42) (which had been redistilled in silica). The digestion was carried out in accordance with the method described by Piper (11). When the digestion was complete, the digest was made up to a suitable volume with distilled water, and the concentration of

[1] Received March 4, 1964.

sodium determined with the atomic absorption instrument. Digests from material containing only very low concentrations of sodium were made up to small volumes so that sodium would be at a concentration high enough to be determined with the atomic absorption instrument. It was found that the response of the instrument to known concentrations of sodium in these highly concentrated solutions was less than in water. The depression in the response to sodium in the solution was found to be attributable to the amounts of sulfuric acid used in the digestion of the plant material and calibration curves were prepared using similar amounts of sulfuric acid.

Purification of Water. Rainwater containing approximately 87 μeq/liter (2 ppm) of sodium was passed through a commercial deionizer consisting of columns of cation and anion exchange resins arranged in series. Treated water contained 3.5 μeq/liter (0.08 ppm) of sodium. This water after distillation in a silica still contained less than 0.0087 μeq/liter (0.0002 ppm) of sodium and was stored in stoppered polythene containers.

Purification of Air. In a preliminary experiment carried out in a glasshouse of conventional design, analysis of plant organs and culture solutions at the end of the experiment showed an increase of about 294 μeq of sodium above the amounts supplied in the culture solution and seeds (table I). This suggested that cultures had received sodium possibly as cyclic salt from the atmosphere. This possibility was tested by placing 4 filter papers horizontally in different sites within the glasshouse, and determining the increase in sodium of each paper at the end of each week of exposure when the papers were replaced by another set. The mean amount of sodium collected per cm^2 per week during the whole period was 0.02 μeq. Subsequent experiments were conducted in a small greenhouse designed to prevent contamination of plants and their cultures by sodium from the atmosphere. A slightly positive pressure was maintained within the greenhouse by a compressor which supplied air continuously to both the cultures for aeration and to the greenhouse itself, through a series of Whatman No. 1 filter papers and washed absorbent cotton wool contained in metal cylinders.

The amount of sodium known to have been present in the culture solution, water and seeds of cultures to which no sodium had been intentionally added was approximately the amount of sodium recovered in the culture solution and plant organs at the end of an experiment in this greenhouse (table I). No increase in the amount of sodium could be detected after the cultures had remained in the experimental greenhouse for 49 days.

All air entering the compressor was drawn through Whatman No. 1 filter papers which were changed at 24 hourly intervals and the sodium they had trapped determined. The amounts of sodium trapped per day (from about 10^5 liters of air) rose and fell periodically (see fig 1). It was found that the amounts were greatest when strong winds blew from the west (the seaward side). Under these conditions opening of the cabinet and manipulation of cultures was avoided.

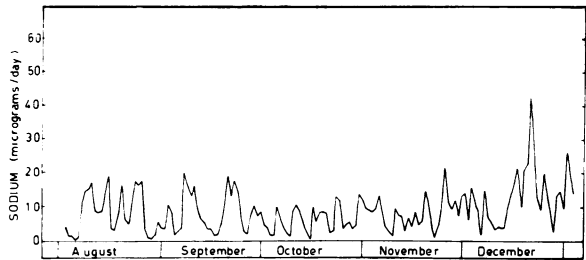

FIG. 1 Amounts of sodium trapped per day from air drawn through a Whatman No. 1 filter paper; about 10^5 liters of air passed through the filter per day.

Table I. *Sodium Supplied; Sodium Recovered*

Conditions of experiment	Amount supplied (μeq)				Amount recovered (μeq)				
	Seeds	Culture solution	Water†	Total	Leaves	Stems and petioles	Roots	Remaining in culture Solution	Total
In conventional glasshouse*	3 (10 Seeds)	43***	80	126	153	33	117	117***	420
In pressurized cabinet**	1.07 (4 Seeds)	5***	0.04	6.11	1.52	1.64	1.66	1.13***	5.95

* Ten plants of *Atriplex vesicaria* were grown in 4.5 liters of basal culture solution to which no sodium had been intentionally added. Experiment was of 93 days duration.

** Four plants of *Atriplex vesicaria* were grown in 2 liters of basal culture solution to which no sodium had been intentionally added. The experiment was of 48 days duration.

*** These data were obtained using emission flame photometry. Due to positive interference from ions in the culture solution, these values are generally higher than they should be.

† Water used in the experiment in the glasshouse contained 4 μeq Na/liter and water used in the pressurized cabinet contained 0.010 μeg Na/liter. Amounts of sodium were calculated on the total volume of water supplied to the culture during the experiment.

Preparation of Nutrient Salts Containing only Minute Amounts of Sodium. The following procedures were used in the preparation of nutrient salts containing only the smallest contaminations of sodium. In table II, concentrations of sodium in solution of purified salts and untreated salts are compared.

Calcium Nitrate: About 700 g calcium salicylate were prepared by slowly adding 500 g salicylic acid and 200 g calcium carbonate simultaneously, with rapid stirring to 2 liters of distilled water at approximately 95°. The resulting solution was heated with rapid stirring until the evolution of CO_2 ceased, and was then filtered, while still hot, through Whatman No. 1 filter paper at a reduced pressure. The filtrate, run into 800 ml silica evaporating dishes, was placed in a refrigerator overnight. The calcium salicylate was recrystallized a further 8 times. The crystals obtained were transferred to a platinum vessel and heated strongly over a bunsen burner. After a short time, the calcium salicylate burst into flames, leaving black ashes. These were then placed in a muffle furnace and heated for about 5 hours at 500°. When the contents of the vessel had been converted to a white powder of calcium oxide free from particles of carbon, the vessel was placed in a desiccator and cooled to room temperature. This was quickly weighed and an equivalent amount of HNO_3 (which had been redistilled in silica) was slowly added to the oxide to form a solution of calcium nitrate. A slight excess of calcium oxide was added to ensure complete neutralization of the acid. The concentration of calcium of this solution was compared with that of a standard solution of calcium nitrate by flame photometer. The solution, diluted to give a concentration 400 times that required in the full culture solution was stored in a stoppered polythene bottle.

Potassium Nitrate: About 100 to 400 g of KNO_3 to be purified was dissolved in twice distilled water in a silica vessel to give an almost saturated solution at 90°. After filtering the solution through Whatman No. 1 filter paper held in a polythene funnel into a silica evaporating dish, the solution was cooled to room temperature and placed in a refrigerator overnight. The crystals formed were washed quickly with chilled distilled water, after the supernatant fluid had been discarded. The recrystallization process was repeated up to 12 times without further filtration and the resulting crystals were dried in an oven at 60° and placed in a desiccator.

Potassium Dihydrogen Phosphate: KH_2PO_4 was recrystallized 6 times by the procedure described for the recyrstallizations of KNO_3.

Diammonium Phosphate: About 80 ml of phosphorus oxychloride (b.p. 105°) was redistilled (in silica) and collected in a platinum vessel. The amount of phosphorus oxychloride equal to a third of its molecular weight (51.13 g) was weighed out and an equivalent amount of twice distilled water (in silica) was cautiously added with a silica pipette. The resulting solution was then boiled with slight excess of water until no further HCl was evolved. The normality of the phosphoric acid produced was found to be 3.77 by carrying out a potentiometric titration of an aliquot against a standardized solution of NaOH. By the addition of 31.8 ml of the 3.77 N phosphoric acid to 6 ml of 6.67 N ammonium hydroxide (redistilled in silica). 10.55 g of diammonium phosphate was formed. When this was made up to 100 ml it had a concentration 400 times as great as that in the full concentration culture solution and had a pH of 8.10.

Magnesium Sulfate and Manganese Sulfate: $MgSO_4$ was recrystallized 6 times and $MnSO_4$ 5 times by the procedure described for KNO_3.

Boric Acid: A saturated solution of boric acid in ethanol (redistilled in silica) was made up in the boiling flask of a silica still. The boron was vola-

Table II. *Sodium Contributed to the Culture Solution as Impurities of Component Salts before and after Purification*

Salt	Conc. of salt in culture solution (μM)	Sodium contributed to culture solution by component salts (μeq/2-1 culture)	
		Untreated analytical reagent salts	Prepared salts
Calcium nitrate	4,000	4.35	0.0174
Potassium nitrate	5,000	0.52	0.0109
Potassium dihydrogen phosphate	1,000	2.18	0.0174
Diammonium phosphate	1,000	0.52	0.0347
Magnesium sulfate	1,000	0.26	0.00174
Boric acid	46	0.0026	0.00087
Manganese sulfate	9.1	0.0252	0.01320
Copper sulfate	0.31	0.00026	0.00026
Zinc sulfate	0.76	0.0065	0.000435
Ammonium molybdate	0.10	0.00022	0.000218
Ferric ammonium ethylene tetra acetate	90	1.39	0.0347
Ammonium chloride	350	0.00569	0.00565
Total sodium in culture solution due to sodium impurities of all component salts		9.26	0.137

tilized as the ethyl ester of boron and the distillate collected in a platinum vessel. After slowly drying the distillate in a water bath, boric acid remained. This was placed in a desiccator until its weight was constant, then made up into a stock solution 10,000 times as concentrated as it was in the final solution. The concentration of sodium was reduced from 435 meq/Kg (10,000 ppm) in boric acid (to which sodium had been previously added) to less than 218 μeq/Kg (5 ppm) in boric acid purified in this way.

Copper Sulfate, Zinc Sulfate, and Ammonium Molybdate: Solutions of copper sulfate, zinc sulfate, and ammonium molybdate were made up from A. R. grade salts 20,000 times as concentrated as they were required in the final culture solution without purification as the amounts of sodium they contributed to the culture solutions were extremely small.

Iron: Iron was supplied to cultures in a single addition of ferric ammonium ethylene diamine tetra acetate. This was prepared by a method similar to that of Jacobson (7) for the preparation of ferric potassium ethylene diamine tetra acetate except that ammonium hydroxide was used in the place of potassium hydroxide in an equivalent amount. KOH, which contained much sodium as an impurity would have been difficult to purify, whereas the ammonium hydroxide redistilled in silica contained an amount of sodium too small to be detected. Other compounds used in the preparation of ferric ammonium ethylene diamine tetra acetate were purified by the following methods. Ferrous sulfate was recrystallized 6 times from solutions acidified with small quantities of H_2SO_4, and the resulting crystals were dried in an oven at 50°. Ethylene diamine tetra acetic acid was dissolved in 2 N ammonium hydroxide (redistilled in silica) and then precipitated by the addition of 2 N HCl (redistilled in silica). This procedure was repeated 4 times and the resulting precipitate was washed in several changes of distilled water and dried in an oven at 50°.

Ammonium Chloride: NH_4Cl was formed by the addition of ammonium hydroxide to an equivalent amount of HCl (both redistilled in silica). The resulting solution was concentrated by boiling, cooled to room temperature, and placed in a refrigerator overnight. The ammonium chloride crystals formed were dried in a desiccator to constant weight and made up in a stock solution 20,000 times the concentration required in the full concentration culture solution.

Culture Apparatus. Seeds were germinated on nylon gauze sewn onto a circle of polythene tubing to form a flat disc which was supported by polystyrene legs in a circular polythene vessel. Water or culture solutions in this vessel were aerated through a fine bore silica tube.

Culture vessels of 2-liter capacity were made from half-gallon polythene containers by cutting off their tops.

The vessels had covers of black or grey Perspex which held 4 evenly spaced plants, secured by white terylene (equivalent to Dacron) fibres washed in many changes of silica distilled water, clamped between split corks made from polythene tubing. Cultures were aerated continuously, with air filtered through cotton-wool and bubbled through frequently changed distilled water and filter papers, by means of centrally placed silica tubes dipping to the bottom of culture vessels.

Paper, black on 1 side and white on the other, was wrapped around each culture vessel to exclude light from the culture solution and roots of the plants.

Samples of all materials associated with the cultures were boiled in small amounts of concentrated HNO_3 (redistilled in silica); the amounts of sodium extracted by this drastic treatment were small in all cases.

Composition of Culture Solution. The composition of the basal culture solution, expressed in μmoles/liter was as follows: KNO_3, 5,000; $Ca(NO_3)_2$, 4,000; $MgSO_4$, 1,000; $(NH_4)_2$ HPO_4, 1,000; KH_2PO_4, 1,000; H_3BO_3, 46; $MnSO_4 \cdot 7H_2O$, 9.1; $CuSO_4 \cdot 5H_2O$, 0.31; $ZnSO_4 \cdot 7H_2O$, 0.76; $(NH_4)_6$ Mo_7 $O_{24} \cdot 4H_2O$, 0.1; NH_4Cl, 350. Iron was supplied as the ferric ammonium ethylene diamine tetra acetate (see above) at 90 μmoles/liter in the basal culture solution.

Procedure. Seeds of *Atriplex vesicaria* were removed from their bracteoles and washed in many changes of distilled water until the amount of sodium in the wash water could not be detected with the flame photometer (adjusted to its maximum sensitivity); the seed then contained a mean amount of 0.27 μeq of sodium per seed. The washed seeds were germinated on distilled water in the seedling culture. After the emergence of the radicle, the distilled water was replaced by the basal culture solution of one-fifth full concentration. When 11 to 14 days old, the seedlings which had acquired cotyledons and apical buds were selected for uniformity and transferred to the polythene culture vessels containing 2 liters of culture solution. Different treatments were applied at this stage.

At harvests, the tops of plants were removed by severing the hypocotyl at the level of the top of the cork. The tops were rapidly separated into leaf-blade and stem and petiole fractions, which were weighed immediately to obtain their fresh weight. The roots were removed from the culture vessels, and, after they had been dried between cleansing tissues, they were dried on aluminum foil trays (previously washed in distilled water and dried) placed in a well-ventilated oven at 95°. After 36 hours the various fractions were cooled in a desiccator to room temperature and their dry weights obtained.

Results

Experiment A. Effect of Small Graduated Amounts of Sodium on Growth of Atriplex vesicaria.

Table III. *Yields of Atriplex vesicaria following the Application of the Sulfates of Sodium and Potassium*

All values are the means of yields from 4 2-liter cultures of 4 plants each. The statistical treatment of total dry weight data was as follows: II > I at 1 % level of significance; III > I at 0.1 % level of significance; III > II at 5 % level of significance.

Treatment	Fr wt/vessel (g) Leaf-blades	Stems and petioles	Roots	Total	Dry wt/vessel (g) Leaf-blades	Stems and petioles	Roots	Total
I. No addition	0.301	0.021	0.221	0.543	0.0324	0.0042	0.0153	0.0519
II. 0.02 meq/liter Na_2SO_4	2.101	0.144	1.441	3.686	0.2354	0.0212	0.0913	0.3479
III. 0.10 meq/liter Na_2SO_4	2.926	0.224	2.148	5.298	0.3468	0.0324	0.1357	0.5149
IV. 0.60 meq/liter Na_2SO_4	2.940	0.228	2.940	6.108	0.3475	0.0355	0.1286	0.5116
V. 0.60 meq/liter K_2SO_4	0.436	0.019	0.326	0.781	0.0433	0.0031	0.0206	0.0670

Different treatments were applied to the cultures in each of 4 blocks (table III).

The concentration of sodium in the full concentration of culture solution due to the sodium contributed by potassium sulfate was reduced from 7.1 to 0.039 µeq/liter by recrystallizing the potassium sulfate 5 times. The cultures within each block were placed in random positions at the beginning of each experiment.

By the twenty-fifth day after germination, plants which had not received sodium sulfate could be distinguished from those which had, by their yellow color and fewer leaves each of smaller area. White necrotic areas appeared along tips and margins of the cotyledons and older leaves on the thirtieth day. Some plants died by the thirty-fourth day. A plant which showed symptoms just described and another which had died, were examined by plant pathologists at the Waite Agricultural Research Institute for the presence of pathogenic organisms. None were found in these plants. When harvested on the forty-eighth day, plants which had received sodium sulfate appeared markedly different from those which had not, having many more leaves of darker green colour which showed no necrosis (fig 2).

The difference between the root systems of plants grown with and without the addition of sodium sulfate was observable at a very early stage (fig 3).

From the results shown in table III obtained when plants were harvested on the forty-eighth day, the yield is seen to have increased asymptotically with increasing sodium sulfate. As plants which received 0.60 meq/liter of potassium sulfate, a concentration equivalent to the highest concentration of sodium sulfate treatment in their cultures, could not be distinguished from the plants grown in the "no addition" cultures, it was evident that the increase in yield with increasing sodium sulfate was not due to the sulfate but to the sodium of the sodium sulfate treatment. This also showed that the part played by sodium in the nutrition of *Atriplex vesicaria* could not be performed by additional potassium when supplied in an amount equivalent to the highest sodium sulfate treatment. The lowest sodium sulfate treatment for maximum dry weight production was about 0.2 meq 2-liter culture and the leaf material contained about 80 µeq/g (dry basis). Although these data would be expected to vary marked-

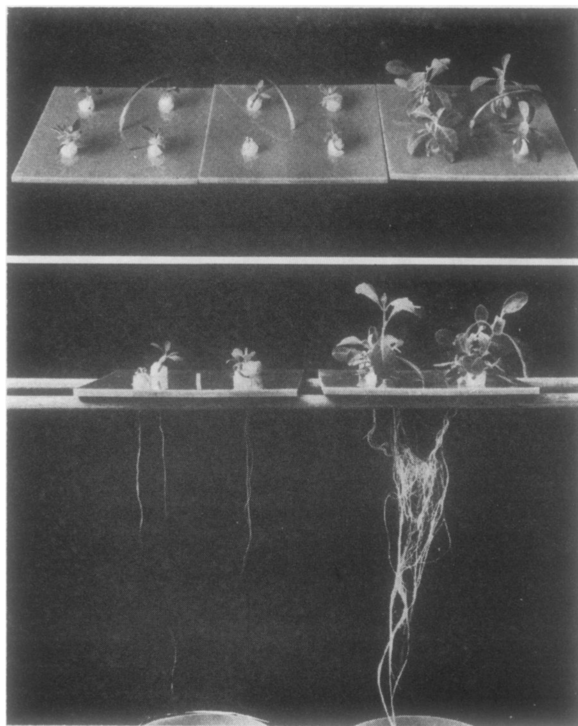

FIG. 2 Comparison between the growth of tops of plants of *Atriplex vesicaria* growing in the basal culture solution with the addition of 0.60 meq/liter potassium sulfate (left), with no addition (center) and with 0.02 meq/liter sodium sulfate (right). The plants had a height of approximately 2.5 cm (left), 2.5 cm (center) and 5.1 cm (right). Photograph was taken on the forty-eighth day.

FIG. 3. Comparison between the top and root growth of plants grown in the basal culture solution which received no addition (left), and 0.60 meq/liter sodium sulfate (right). The heights of the tops of the plants were approximately 2.5 cm (left) and 6.4 cm (right). The photograph was taken on the forty-eighth day.

ly according to the conditions of the experiment, the sodium requirements by *Atriplex vesicaria* were high in comparison with the requirements of micronutrients by plants of other species (17).

Recovery of Sodium Deficient Plants of Atriplex vesicaria Following the Application of Sodium. Fourteen days after germination, plants selected for uniformity were transferred from seedling cultures to culture vessels containing the basal culture solution without added sodium. On the sixteenth day sodium sulfate (0.10 meq/liter) was added to 1 set of culture vessels and 7 days later plants growing in these cultures could be distinguished from the controls by their darker green color. Symptons similar to those obtained in the previously described experiment again appeared in cultures which had not received sodium, and by the thirty-first day, when a second set of deficient cultures received a treatment of 0.10 meq/liter of sodium sulfate, symtoms were severe.

Four days after receiving this delayed sodium treatment, plants showed signs of recovery by a progressive change of color in older leaves (and in some cases cotyledons) from yellow to green; greening commenced at tips and around midribs, and gradually spread over the laminae.

Plants growing in the set of cultures which received no sodium treatment throughout the experiment became progressively more chlorotic, making little further growth. On the other hand, marked growth occurred in both sets of cultures which received added sodium. When harvested on the forty-ninth day, the results in table IV were obtained.

The complete recovery of plants growing in cultures which received a small addition of sodium sulfate (even though they were adequately supplied with sulfate), is convincing evidence for sodium being an essential nutrient element for *Atriplex vesicaria*.

Experiment B. Effects of Lithium, Sodium, Potassium or Rubidium on Sodium-Deficient Plants of Atriplex vesicaria. On the fifteenth day after germination, seedlings selected for uniformity were transferred from seedling cultures to culture ves-

Table IV. *Fresh and Dry Weight Changes after Recovery of Sodium Deficient Plants of Atriplex vesicaria following the Application of Sodium Sulfate Treatments*

All values are the means of yields of 2 vessels of 4 plants each. The statistical treatment of total dry weight data was as follows: III > I at 1 % level of significance; II > I at 0.1 % level of significance; II > III at 5 % level of significance.

Treatment	Fr wt/vessel (g) Leaf-blades	Stems and petioles	Roots	Total	Dry wt/vessel (g) Leaf-blades	Stems and petioles	Roots	Total
I. No sodium sulfate	0.877	0.078	0.671	1.626	0.0894	0.0124	0.0481	0.1499
II. 0.10 meq/liter Na_2SO_4 applied on day 16	8.139	1.007	6.923	16.069	1.0236	0.1619	0.4810	1.6665
III. 0.10 meq/liter Na_2SO_4 applied on day 31	3.713	0.334	2.800	6.847	0.4713	0.0544	0.2133	0.7390

Table V. *Effects of Equivalent Amounts of the Sulfates of Lithium, Sodium, Potassium or Rubidium when Applied to Cultures of Sodium-deficient Plants of Atriplex vesicaria*

Plants were harvested on the forty-fourth day. The statistical treatment of total dry weight data was as follows: III > I, II, IV, V at 0.1 % level of significance; I, II, IV, V, indistinguishable.

Treatment	Sodium/2–1 culture (μeq) As impurity of basal culture solution	Due to treatment salts	Total	Yield (Each value is the mean of 4 replications) Mean dry wt/culture (g) Leaf-blades	Stems and petioles	Roots	Total
I. Control	0.14	(No addition)	0.14	0.179	0.030	0.061	0.270
II. Li_2SO_4 0.10 meq/liter	0.14	0.052 (Impurity)	0.192	0.163	0.027	0.051	0.241
III. Na_2SO_4 0.10 meq/liter	0.14	200 (Treatment)	200.14	0.761	0.183	0.288	1.232
IV. K_2SO_4 0.10 meq/liter	0.14	0.017 (Impurity)	0.157	0.169	0.024	0.049	0.242
V. Rb_2SO_4 0.10 meq/liter	0.14	0.069 (Impurity)	0.209	0.220	0.045	0.071	0.336

sels which were placed in the pressurized greenhouse. On the twenty-second day, when symptoms of sodium deficiency were clearly recognizable, 4 cultures each containing 4 plants were harvested. The mean dry weight per culture was 0.0187 ∓ 0.0012 g. On the same day, the differential treatments shown in table V were applied to the cultures of each of 4 blocks. The concentration of sodium in the culture solution due to the sodium associated with the treatment application is also given and was not greater than 0.035 μeq/liter (0.0008 ppm) in any of the treatments other than that of sodium sulfate. On the twenty-seventh day, plants which had received the sodium sulfate treatment showed signs of recovery. Plants growing in cultures which received no sodium treatments became progressively more chlorotic, making little further growth. On the other hand, marked growth occurred in the set of cultures which had received sodium.

Plants in untreated, and in lithium, potassium and rubidium sulfate treated cultures were indistinguishable. By the thirty-third day, some plants had died in the cultures which had not received sodium. The mean dry weights per culture of 4 plants obtained for each treatment on the forty-fourth day are shown in table V.

At the final harvest, the plants in cultures which received sodium sulfate had made about 4 times as much growth as the plants in the cultures which had received the other treatments. The results of this experiment show clearly that the essential function of sodium in the nutrition of *Atriplex vesicaria* cannot be performed by equivalent amounts of lithium, potassium, or rubidium. This finding is similar to that of Allen and Arnon (1) who showed that the blue-green alga, *Anabaena cylindrica* Lemm. has a specific requirement for sodium in its nutrition which is not satisfied by supplying any of the other group 1 elements in the same concentrations.

Uptake and Distribution of Sodium and Potassium in Plants of Atriplex vesicaria. The effects of graduated amounts of sodium sulfate on dry weight production and on the concentrations of sodium and potassium in leaves, stems and roots of *Atriplex vesicaria* are shown in table VI. Seedlings were transferred to cultures 9 days after germination, the different treatments applied on the tenth day, and the plants harvested on the fifty-first day.

The dry weight production increased asymptotically with increasing applications of sodium sulfate. The concentrations of sodium increased strikingly in all fractions, especially in the leaves where the increase was more than 100-fold when 0.60 meq/liter of Na_2SO_4 was supplied.

The concentrations of potassium in leaves, stems, and roots increased when 0.01 meq/liter of Na_2SO_4 was supplied, but decreased when the amounts of sodium sulfate were further increased to 0.60 meq/liter.

Discussion

Experiments have been described in which sodium was shown to be an essential nutrient element for *Atriplex vesicaria* according to the criteria of Arnon and Stout (2). Plants, protected from atmospheric contamination of sodium, grown in culture solutions containing only small amounts of sodium showed characteristic deficiency symptoms by the yellowing of their leaves and development of white necrotic patches on their tips and margins. Plants developed few or no secondary shoots and in some cases died at an early stage; no pathogenic organisms could be found in their tissues. Thus, the first of the criteria of Arnon and Stout (2), viz., "a deficiency of it makes it impossible for the plant to complete its life cycle," was satisfied.

In the second experiment described, sodium-deficient plants recovered after an application of a small amount of sodium which had been delayed until the onset of severe symptoms; recovery took about a week.

Table VI. *Effects of Treatments of Sodium Sulfate on Dry Weight Production and Concentrations of Sodium and Potassium in Leaves, Stems and Roots of Atriplex vesicaria*

The statistical treatment of total dry weight data was as follows: VI, V, IV, III, II > I at 0.1 % level of significance; VI > V at 5 % level of significance; II, III; III, IV; IV, V indistinguishable.

Treatment	Dry wt (g) All values are the mean of 5 cultures of 4 plants each				Conc. of sodium and potassium (meq/Kg) All values are the means of duplicate samples taken from 5 replicated cultures of each treatment					
					Sodium			Potassium		
	Leaves	Stems	Roots	Total	Leaves	Stems	Roots	Leaves	Stems	Roots
I. No sodium sulfate	0.0560	0.008	0.022	0.086	10.0	7.1	2.6	2,834	1,913	1,547
II. 0.01 meq/liter Na_2SO_4	0.257	0.043	0.098	0.398	47.8	6.5	6.5	4,450	2,583	1,442
III. 0.02 meq/liter Na_2SO_4	0.377	0.066	0.138	0.581	78.3	11.7	7.0	2,504	2,197	1,563
IV. 0.06 meq/liter Na_2SO_4	0.461	0.088	0.173	0.722	213.0	20.2	11.7	2,476	2,169	1,540
V. 0.10 meq/liter Na_2SO_4	0.489	0.089	0.193	0.771	295.7	51.0	29.1	2,225	2,205	1,683
VI. 0.60 meq/liter Na_2SO_4	0.685	0.149	0.267	1.101	1,129	338.7	257.8	1,688	1,934	1,445

The results of the third experiment showed that of the group 1 elements, lithium, sodium, potassium or rubidium only sodium brought about the recovery of sodium-deficient plants of *Atriplex vesicaria*. The plants receiving no sodium additions to their cultures could not be distinguished from those receiving the treatments of lithium, potassium or rubidium. It appeared, therefore, that the second of the criteria, viz., "such deficiency is specific to the element in question and can only be prevented by supplying this element," has almost certainly been satisfied.

The fulfillment of the third of the criteria (2), viz., "the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil medium," is difficult to achieve. However, plants in these experiments were grown in solution culture so that the sodium supplied in the treatments was more likely to have exerted its effects directly in the nutrition of the plant than if more complicated media had been used. Even so the possibility still exists that the sodium corrected an unfavorable chemical or microbiological condition of the culture solution, and this possibility cannot be dismissed until a specific essential role of sodium in the metabolism of the plant has been demonstrated. These experiments were of short duration so that the possible complicating effects due to the depletion of nutrients in the culture solution were avoided, and the risk of the heavy infection of the cultures by organisms such as algae, fungi and bacteria were minimized.

The potential sources of sodium to the plant are the culture solution, water, culture vessels, air in greenhouse and seed, which all require further critical examination if very low sodium conditions are to be achieved in cultures.

The salts of the culture solution contribute less than 0.07 μeq/liter (0.0016 ppm) of sodium to the solution. This amount is calculated from the sum of the impurities of sodium remaining in the concentrated solutions of the individual salts of the culture solutions. These were estimated with an atomic absorption instrument, which was working in some cases at the limits of its sensitivity. To make further progress in the purification of certain salts a more sensitive instrument would be needed to determine the efficiency of the procedures used.

The culture vessels appeared satisfactory as the amounts of sodium they contributed to the culture solution could not be detected, nor could any increase in the sodium in the water they contained be detected after open culture vessels had remained in the pressurized greenhouse for a fortnight. However, the concentration of sodium in water in a culture vessel after a fortnight of continuous aeration even when covered, increased by 0.4 μeq/liter (0.01 ppm). At this rate of contamination, the amount of sodium in the most highly purified solution used in this study would be increased many times in even a short experiment. However, in subsequent experiments the air used for aeration was effectively freed from sodium by passing it through distilled water contained in a train of plastic vessels.

Water from the silica still contained less than 0.0087 μeq/liter (0.0002 ppm) of sodium: this is low compared with the concentration of sodium in the culture solution and would not be an important source of sodium except in an experiment in which a large amount of water was used. The amount of sodium contributed to a culture by the seeds of *Atriplex vesicaria* was approximately 0.27 μeq per seed after washing in several changes of distilled water.

Summary

Methods are described by which plants were grown under conditions from which sodium was carefully minimized. Culture solutions prepared from purified salts contained less than 0.07 μeq/liter (0.0016 ppm) of sodium as an impurity and water contained less than 0.0087 μeq/liter (0.0002 ppm) of sodium.

Cultures were protected from atmospheric contamination by sodium in a small greenhouse maintained at a slightly positive pressure by a continuous supply of filtered air. Under these conditions it was not possible to detect any increase in the amount of sodium in a culture or its plants over the period of an experiment.

Characteristic deficiency symptoms developed on about the twentieth day by plants of *Atriplex vesicaria* Heward ex Benth. (Bladder salt bush) which had not received an application of sodium to their cultures. Leaves became chlorotic and developed necrotic patches at their tips and along their margins after which little further growth was made. By about the thirty-fifth day some plants died. Plants receiving 0.02 meq/liter (0.46 ppm) Na_2SO_4 made favorable growth and when harvested on the forty-eighth day had approximately 10 times the dry weight of plants which had not received sodium. Plants which had developed severe symptoms of sodium deficiency recovered within about 7 days of receiving an application of sodium to their culture solutions.

Only sodium of the group 1 elements effected the recovery of sodium-deficient plants of *Atriplex vesicaria*. Plants receiving equivalent amounts of lithium, potassium or rubidium in their cultures could not be distinguished from those to which no addition of sodium had been made.

It is concluded that small amounts of sodium are essential for the growth and development of *Atriplex vesicaria*.

Acknowledgments

The author is grateful to the late Professor J. G. Wood for the encouragement and advice he gave throughout this investigation.

Dr. R. L. Specht and Miss Margaret E. Jackman gave constructive criticism and practical assistance; Dr. A. Walsh and Mr. G. F. Box of the Division of Chemical Physics, C.S.I.R.O. especially designed and constructed an atomic absorption apparatus which made possible precise determinations of sodium in solutions containing high concentrations of other ions. Mr. A. F. Jenkinson carried out statistical analyses and figures 2 and 3 were prepared by Mr. K. P. Phillips of the Waite Agricultural Research Institute. Grateful acknowledgment is given for this help.

This work was made possible by grants from the University of Adelaide and the Commonwealth Government of Australia.

Literature Cited

1. Allen, M. B. and D. I. Arnon. 1955. Studies on nitrogen-fixing blue-green algae. II. The sodium requirement of *Anabaena cylindrica*. Physiol. Plantarum 8: 653–60.
2. Arnon, D. I. and P. R. Stout. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. 14: 371–75.
3. Box, G. F. and A. Walsh 1959. C.S.I.R.O. Ind. Res. News No. 17.
4. Brownell, P. F. and J. G. Wood. 1957 Sodium as an essential micronutrient element for *Atriplex vesicaria* Heward. Nature 179: 653–56.
5. Harmer, P. M. and E. J. Benne. 1945. Sodium as a crop nutrient. Soil Sci. 60: 137–48.
6. Harmer, P. M., E. J. Benne, W. M. Laughlin, and C. Key. 1953. Factors affecting crop response to sodium as applied as common salt to Michigan Muck Soil. Soil Sci. 76: 1–17.
7. Jacobson L. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene diamine tetra-acetate. Plant Physiol. 26: 411–13.
8. Lehr, J. J. 1949. Exploratory pot experiments on sensitiveness of different crops to sodium. A. Spinach. Plant Soil 2: 37–48.
9. Lehr, J. J. 1953. Sodium as an essential plant nutrient. J. Sci. Food Agr. 4: 460–71.
10. Lehr, J. J. 1957. Is sodium an essential element for plants? Chilean Nitrate Agricultural Service, London. Information No. 30.
11. Piper, C. S. 1950. Soil and Plant Analysis. Interscience Publishers, New York.
12. Riceman, D. S. and G. B. Jones. 1958. Distribution of zinc and copper in subterranean clover (*Trifolium subterraneum* L.) grown in culture solutions supplied with graduated amounts of zinc. Australian J. Agr. Res. 9: 73–122.
13. Richards, F. B. 1944. Mineral nutrition of plants. Ann. Rev. Biochem. 13: 611–30.
14. Williams, M. C. 1960. Effect of sodium and potassium salts on growth and oxalate content of *Halogeton*. Plant Physiol. 35: 500–05.
15. Wood, J. G. 1925. The selective absorption of chlorine ions; and the absorption of water by the leaves of the genus *Atriplex*. Australian J. Exptl. Biol. Med. Sci. 2: 45–56.
16. Woolley, J. T. 1957. Sodium and silicon as nutrients for the tomato plant. Plant Physiol. 32: 317–21.
17. Wybenga, J. M. 1957. A contribution to the knowledge of the importance of sodium for plant life. In: Investigations with Radioactive Sodium. Kantoordruk de Goede, Wageningen, Holland.