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J. H. LONGWELL, Director

# The Uptake of Ca and K By Young Soybean Plants

W. T. HIGDON AND C. E. MARSHALL



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This bulletin reports on Department of Soils  
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## SUMMARY

Soybeans were grown for 20 days on 24 liquid nutrient media comprising chloride and bicarbonate solutions, colloidal Putnam clay, colloidal Wyoming bentonite, and a suspension of a carboxylic cation exchange resin (Amberlite XE64.) The variables under study were: absolute activity levels of  $K^+$  and  $Ca^{++}$  ions, the ratio  $K/\sqrt{Ca}$ , and the free energy change in the exchange reaction between plant root and cationic medium.

At the beginning of the treatments all seedlings were strongly calcium-deficient. Hence the study involves chiefly the conditions for recovery from marked calcium deficiency. The cation exchange properties of the roots, composition of roots and tops as regards K and Ca, and final yields were used as criteria.

The evidence from cation exchange experiments on plant roots indicated clearly that simple Donnan equilibrium relationships did not adequately account for the results. Consideration of plant composition and uptake in relation to  $K/\sqrt{Ca}$  ratios for the substrates also brought out this point. In recovery from calcium deficiency calcium was found necessary for the efficient utilization of potassium. The absolute level of the calcium activity was found to be of dominant importance. The influence of the free energy change in the exchange reaction between plant roots and substrate was clearly apparent in the chloride-bicarbonate comparisons. In the colloidal systems superiority of uptake of Ca and K over true solutions could not be decisively linked to any of the above factors.

# The Uptake of Ca and K By Young Soybean Plants

## INTRODUCTION

It has long been recognized that the uptake of the major metallic nutrient elements of plants is affected and perhaps even governed by relationships between their concentrations or activities in nutrient media. Thus calcium affects potassium uptake and vice versa. These effects were first demonstrated in the mutual effects of monovalent and divalent cations, although they are also found for cations of the same valency but having different functions within the plant; for example, calcium and magnesium. The use of the term "ionic antagonism" to describe these effects is now decreasing. More and more attention is being focussed on individual mechanisms, both outside and within the plant, to explain the overall effects.

The present study is the third of a series in which soybeans have been grown in a variety of nutrient media comprising both true solutions and colloidal systems. In the first of these (10) five comparisons were made between bicarbonates and Wyoming bentonite systems using different proportions of calcium and potassium as well as different individual cationic activities. The Wyoming bentonite systems considerably outyielded the bicarbonates as regards dry matter, but the composition of the tops was almost the same with respect to calcium and potassium, and it remained practically constant through various proportions of calcium and potassium.

In the second study (13, 14, 21) chlorides, bicarbonates and suspensions of Amberlite IR 120 (a sulphonic acid resin exchanger) were used in mixed K-Ca systems; these, together with Putnam clay and Wyoming bentonite, were employed in single K and Ca systems. Here the importance of the free energy change in the exchange reaction between roots and substrate was first recognized, although other factors also influenced the results.

The present series was designed to throw light on four possible external factors as they affect potassium and calcium uptake: (1) activity of nutrient ions,

(2) free energy change in the exchange reaction between plant root and nutrient medium, (3) cationic ratios in the nutrient medium (4) possible effects of contact exchange between substrate and root.

Regarding internal factors, it was anticipated that mineral analyses of the plants would facilitate comparisons with conclusions drawn from modern theories of uptake. The transition from strictly external to strictly internal factors was to be covered by studies of the cation exchange properties of soybean roots grown under the different treatments.

## DISCUSSION OF RELEVANT LITERATURE

In its interaction with nutrient solutions the growing plants acts as a highly selective concentrator of metallic elements. The same crop grown to the same stage in a variety of nutrient media usually shows a considerable range in which the total metallic elements present, expressed in equivalents per gm. of dry matter, remain fairly constant (Van Itallie 8, Russell 18). This relationship does not extend over the region of extreme deficiency in a major nutrient element, nor over the region of luxury consumption of a particular element such as potassium (Marshall 11). It can be regarded as a gross expression of the sum of the linkages between uptake of metallic elements and growth, to be taken only as a crude approximation (Hoagland 7). It does not lend itself to deductions regarding mechanics of uptake.

When a single metallic element is investigated, under conditions which ensure an adequate supply of all other nutrients, the relationship between uptake and external concentration of the given element follows a typical exponential growth curve (Hoagland 7. p. 155) However, in the range of approximate constancy as regards total cations, the increase in concentration of one element parallels a decrease in the others. (see Hoagland 7. p. 165 for an example in which an increase in K accompanies a decrease in Ca and Mg).

Much attention has been centered upon calcium-potassium relationships in growing plants. Both elements can be concentrated in the plant from extremely dilute nutrient solutions. From the literature one obtains the general impression that more serious injury is caused to young plants by pronounced calcium deficiency than by similar degrees of potassium deficiency. This may be connected with the fact that many seeds contain much more potassium than calcium. In extreme cases, of course, both deficiencies are fatal.

Certain general ionic relationships have been deduced by the application of Donnan principles to plant roots and their external environments. These can be used only if two conditions are fulfilled. First, the system must be in chemical equilibrium; second, constraints must exist which prevent free diffusion of certain ionic species. The constrained part of the system is usually taken as an im-

mobile colloidal ion which attracts and holds an atmosphere of small ions. In a crude way the soil can be regarded as one colloidal system of this kind, the plant root as another. If necessary, a true solution can be thought of as existing between them. Throughout the whole system at equilibrium there should exist a series of relationships such as:

$$\begin{aligned} \left[ a_{\text{Na}}/a_{\text{K}} \right]_{\text{solution}} &= \left[ a_{\text{Na}}/a_{\text{K}} \right]_{\text{colloid I}} = \left[ a_{\text{Na}}/a_{\text{K}} \right]_{\text{colloid II}} \\ \left[ a_{\text{Mg}}/a_{\text{Ca}} \right]_{\text{solution}} &= \left[ a_{\text{Mg}}/a_{\text{Ca}} \right]_{\text{colloid I}} = \left[ a_{\text{Mg}}/a_{\text{Ca}} \right]_{\text{colloid II}} \\ \left[ a_{\text{K}}/\sqrt{a_{\text{Ca}}} \right]_{\text{solution}} &= \left[ a_{\text{K}}/\sqrt{a_{\text{Ca}}} \right]_{\text{colloid I}} = \left[ a_{\text{K}}/\sqrt{a_{\text{Ca}}} \right]_{\text{colloid II}} \end{aligned}$$

Even though a growing plant is not in chemical equilibrium with its environment, such relationships may exert some influence and will become more controlling the slower the rate of metabolism, provided of course that a constraint on the colloid side effectively exists. As far as the soil system is concerned the situation is similar to that for other negatively charged colloids, and the above ratios should hold. More refined considerations of the distribution of the ionic atmosphere around individual colloidal particles (Gouy theory) make no difference as regards these ionic activity ratios. However, individual ionic activities are greatly affected by the geometry of the electrical double layer.

On the root side similar arguments may be advanced, although it is difficult to obtain proof that Donnan conditions alone dominate the situation, even at root surfaces. Roots continually exude organic acids in addition to carbon dioxide, and a complexing action between such molecules and nutrient cations cannot be excluded. Furthermore, passage from the exterior root surface to the interior might conceivably occur by the molecular transport of chelated cations. Such molecules would not necessarily be very large and might, to some extent, pass into the outer solution, thus vitiating the Donnan condition of a complete constraint. In the interior of plants, quite different considerations dominate the conditions of uptake. Here the concentration ratios are very different from the activity ratios of the external media. This is not surprising when the nutrient cations are considered as components of enzyme systems, or when disturbing factors such as the precipitation of calcium oxalate play a part.

Ideas regarding the application of Donnan principles to the soil as a colloidal system were first brought forward by Mattson in a series of papers beginning in 1929 (15).

The particular form of the monovalent-divalent ion relationship, namely that at equilibrium throughout a Donnan system the ratio  $a_m/\sqrt{a_d}$  should be constant, was taken up in respect of soil-soil solution equilibria by Mattson and Wiklander (17, 1940). Schuffelen and Loosies utilized the Donnan relationship for monovalent cations ( $\text{H}^+$  and  $\text{K}^+$ ,  $\text{H}^+$  and  $\text{Na}^+$ ) to link the external system with the plant root surface (19, 20). They used pH determinations in colloidal

systems and in their equilibrium outer solutions in order to calculate ionic activities in colloidal systems. The latter were used as substrates for plant growth.

A series of papers from Mattson's laboratory demonstrated that diverse plant materials have the properties of ionic exchangers (16). Plant roots were first treated as cationic exchangers by Jenny and Overstreet (9) in their development of the idea of a contact exchange mechanism between root and colloidal medium. This formulation rests on the concept of a kinetic mechanism of surface diffusion and is not a thermodynamic derivation for a system at equilibrium.

The demonstration by Williams and Coleman (22) that living plant roots have a fairly well-defined cation exchange capacity, independent of their rate of metabolism, served to stimulate further work. In particular, Drake et al. (3) linked the cation exchange capacity with potassium-calcium relationship through Donnan theory. In these applications a number of assumptions are involved which are difficult to prove directly. For instance, in connecting cation exchange capacities with calcium/potassium ratios it is necessary to assume that activity coefficients for given cations held by roots are the same for different plant species and for different nutrient substrates.

The link between cations at the root surface and those in the interior of the plant has been discussed by Epstein and Hagan (4) whose experiments on rate processes have led them to a modified concept of the function of cations held at root surfaces. They interpret their experiments in terms of specific sites for each nutrient cation and conclude that these specific sites form only a small fraction of the total sites at which exchange reactions can occur. Thus the larger part of the exchange capacity is taken up by cations not directly metabolized, but which form a reserve.

## GENERAL PLAN

In setting up a series of plant nutrient experiments to study potassium-calcium relationships in young soybean plants, choices have to be made regarding the nature of the nutrient systems, the potassium and calcium concentration and activity levels, and the  $K/\sqrt{Ca}$  ratios. Based on previous experience, it was felt that very low K and Ca activities should be attempted, that a wide range of  $K/\sqrt{Ca}$  ratios should be set up, and that five types of substrates should be used. The five types of substrates were chlorides, bicarbonates, Wyoming bentonite, Putnam clay and the carboxylic exchange resin, Amberlite XE 64.

The concentrations and degrees of saturation of the colloidal systems were therefore chosen with regard to the cation activities and ratios desired. Limitations were caused by the inaccuracies of cation activity measurements at extremely low values and by the requirement that renewal of the nutrient systems should be rapid enough to maintain reasonable constancy in the external environment during the period of most rapid growth. Thus in very dilute systems large volumes of substrates were required.

### CHOICE OF PROCEDURES

Experimentally, choice had to be made between two procedures for the definition of cation activities in colloidal systems. In all previous work from this laboratory, clay membrane electrodes combined with saturated K Cl calomel electrodes have been used. As mentioned earlier, Schuffelen and Loosjes employed the Donnan relationships between colloidal systems and outer solutions. By determining the pH in the colloidal system and the outer solution and the metallic ion concentration in the latter, the activity of the metallic ion in the colloidal system was computed.

The two procedures were compared in two series of experiments: (1) Calcium activities were determined on four concentrations of Putnam clay (90 percent Ca, 10 percent H saturation) using both the clay membrane technique and the Schuffelen and Loosjes method. The latter gave extremely high fractions active, which increased rapidly with clay concentration. The 2 percent and 1 percent clays gave computed fractions active for calcium of 21 percent and 5.4 percent respectively. The potentiometric method using clay membrane electrodes to determine calcium activities in the clay suspensions, gave 0.62 percent and 0.76 percent for Ca at these concentrations, in agreement with previous experience both by potentiometric and conductometric methods. (2) Potassium activities were determined by both methods using a fixed concentration of Putnam clay (2%) and different degrees of saturation with potassium (from 2% to 12%). The potassium activities calculated through the Donnan relationship were higher than the total exchangeable potassium concentration in the clay suspension. The membrane electrode method gave fractions active from 13 to 20 percent in agreement with previous work. We are forced to conclude that the procedure via the Donnan relationships completely fails for this clay suspension.

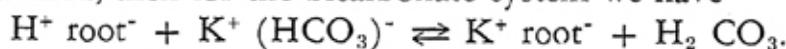
Thus in standardizing colloidal systems for use as substrates we have relied on potentiometric determinations involving glass electrodes for hydrogen ions and clay membrane electrodes for metallic cations. As far as cationic ratios were concerned, equilibration with dilute salt solutions was used.

### CHOICE OF SUBSTRATES

The comparison of chlorides with bicarbonates as substrates for plant growth provides a simple means of illustrating the importance of free energy changes in the interchange of hydrogen ions from the plant root with metallic cations from the substrate. It was used by Marshall and Upchurch (13, 14) in this way. The argument is as follows. If the reaction



is taken as standard, then for the bicarbonate system we have



The low dissociation of carbonic acid drives the second reaction more nearly to completion than the first. One way of expressing this quantitatively is by com-

puting the free energy difference for the hydrogen ion in the two cases, assuming that the root functions are identical. In both chloride and bicarbonate systems one can set up extremely dilute substrates in which the assumption of complete dissociation involves only small errors. If necessary, one could proceed to concentrations below the range of applicability of clay membrane electrodes, although in the present series this was not required.

The two colloidal clays, Wyoming bentonite and Putnam clay have been studied extensively by electrochemical methods; their cationic bonding energy relationships for calcium-hydrogen, potassium-hydrogen, and calcium-potassium systems have been determined (2). Wyoming bentonite was used as a colloidal substrate for soybeans by McLean. Both clays were similarly used by Upchurch. In the latter study the importance of the free energy of the cation exchange reaction between root and clay was clearly recognized in a comparison of dilute calcium chloride and calcium bentonite as substrates.

The choice of Amberlite XE 64 as a colloidal substrate was based on previous experience with Amberlite IR 120 (used by Upchurch) and on a preliminary characterization. In the case of Amberlite IR 120, under the conditions employed in the soybean study, the bonding energies of calcium and hydrogen were both high, so that the free energy change for the reaction as compared with the chloride system was close to zero. This proved informative, since calcium is held very tightly by this sulphonic acid exchanger; hence under many circumstances plants cannot successfully compete for it. However, in this case experience showed that soybeans grew and accumulated calcium from the Amberlite IR 120 and the chloride systems almost equally when the systems were so set up that the respective calcium activities and free energy changes for the calcium-hydrogen interchange were approximately equal.

Amberlite XE 64 (Rohm and Haas) is a carboxylic exchanger which possesses the characteristics of a weak colloidal acid whose salts with monovalent cations show a low bonding energy while those with divalent cations show a moderate bonding energy. Electrochemical studies revealed much similarity between XC 64 and the humic acid fraction of soil organic matter examined by Marshall and Patnaik (12). After dry grinding, XE 64 contained about 6.2 percent of material less than  $2\mu$  equivalent spherical diameter and 18.7 percent less than  $6\mu$  e.s.d. (specific gravity 1.374 for dried resin). A fraction less than  $4\mu$  was separated by sedimentation and used in chemical characterization. For the nutrient substrate material of less than  $6\mu$  diameter was employed.

## CATIONIC RATIOS AND ACTIVITIES

In setting up substrates with known values of the ratio  $K/\sqrt{Ca}$  much preliminary experimentation with the colloidal systems was required; especially with Amberlite XE 64, for which no previous experience was available. Potentiometric and dilute exchange determinations were made on Ca-H and K-H sys-

tems. Mixed Ca-K-H systems also were investigated. Unfortunately, the preliminary characterization took place several months before the substrates were finally prepared in bulk and the soybeans grown on them. During this period the selective clay membrane electrodes which were relied upon for the determination of potassium ion activities in presence of calcium deteriorated and became unusable. Time did not permit of the preparation and standardization of a new batch.

Hence the individual cationic activities in the mixed Ca-K colloidal systems were not determined, and the  $K/\sqrt{Ca}$  ratio for each was actually found by dilute calcium chloride equilibration. In these cases the  $K/\sqrt{Ca}$  ratios of the colloidal systems proved considerably lower than those of the chloride and bicarbonate solutions. It was found in the preliminary experimentation that a four-fold range in  $K/\sqrt{Ca}$  could be covered simultaneously for the three colloids chosen. The selected values were 0.08 and 0.02, and true solutions were made up to correspond; however the actual ratios for the three colloidal systems were closer to 0.04 and 0.01. The range for true solutions was broadened by the addition of both chloride and bicarbonate systems at 0.005 and a single chloride system at 0.001.

The absolute cation activities were selected after careful consideration of previous work. In all the systems containing Ca only, and in the K-Ca bicarbonate and chloride solutions with four exceptions, the calcium activity was set at  $4.0 \times 10^{-5}$ . The mixed K-Ca colloidal systems were not well defined in this respect since the electrodes failed, but from the preliminary work they should be of this same order of magnitude. Values of  $K/\sqrt{Ca}$  of .005 and .001 necessitated the use of more concentrated Ca solutions.

The compositions of the 24 substrates as regards Ca and K are given in Tables 1 and 2.

In Table 1 the cationic ratios are expressed in two ways, namely  $K/\sqrt{Ca}$  and  $RT \ln (K/\sqrt{Ca})$ , the latter being termed by Woodruff the free energy of exchange (22). It is expressed in calories per equivalent. Donnan theory and Gouy theory both require that at equilibrium this quantity should be constant throughout one or more colloidal systems and a true solution in contact. It can be determined experimentally by a small exchange against a dilute salt solution, or by calculation from individual cation activities obtained by the use of selective and non-selective membrane electrodes. The former method was used in characterizing the three colloidal substrates.

In Table 2 calcium, potassium, and hydrogen activities, as determined potentiometrically, have been used to calculate the mean free bonding energy for each cation. The formula is

$$(\Delta F)_{\text{cation}} = RT \ln \frac{c}{a}, \text{ where } c \text{ is the}$$

total concentration of the cation and  $a$  is the measured activity. The chief uncertainty lies in the determination of  $(\Delta F)_H$  since for this purpose it is necessary

TABLE 1--CHARACTERISTICS OF SUBSTRATES

No.	Nature	pH	CATION ACTIVITY moles/liter		K/ $\sqrt{\text{Ca}}$	$\Delta F_{(\text{K}-\frac{1}{2}\text{Ca})}$ cal/equiv.
			Ca.	K.		
1	Ca Chloride	6.22	5.3x10 <sup>-5</sup>			
2	2.2% Ca-H-Putnam	5.28	4.0			
3	0.18% Ca-H-Amberlite XE64	5.72	4.0			
4	0.9% Ca-H-Wyo. Bent.	5.50	4.0			
5	Ca Bicarbonate	6.45	5.4			
6	K Chloride	6.22	--	37.2x10 <sup>-5</sup>		
7	1.0% K-H Putnam	6.50	--	30.0		
8	0.02% K-H Amberlite XE64	6.28	--	30.0		
9	0.60% K-H Wyp. Bent.	6.60	--	30.0		
10	K Bicarbonate	6.85	--	28.9		
11	K-Ca Chloride	6.05	6.8	64.3	0.078	1510
12	K-Ca Bicarbonate	6.85	8.4	52.5	0.057	1690
13	2% Ca-K-H Putnam	5.80	--	--	0.042	1880
14	1.0% Ca-K-H Wyo. Bent.	5.11	--	--	0.042	1880
15	0.20% Ca-K-H Amberlite XE64	6.11	--	--	0.044	1850
16	K-Ca Chloride	5.98	6.2	16.9	0.021	2280
17	K-Ca Bicarbonate	6.58	8.2	12.0	0.013	2560
18	2.0% Ca-K-H Putnam	5.67	--	--	0.011	2670
19	1.0% Ca-K-H Wyo. Bent.	5.55	--	--	0.010	2730
20	0.20% Ca-K-H Amberlite XE64	5.92	--	--	0.011	2670
21	Ca-K Bicarbonate	6.58	82.0	12.1	0.0042	3240
22	Ca-K Chloride	6.00	66.1	16.8	0.0065	2930
23	Ca-K Chloride	6.10	398	35.1	0.0056	3080
24	Ca-K Chloride	6.12	416	10.11	0.0016	3830

TABLE 2--CATIONIC BONDING ENERGIES FOR HOMIONIC SUBSTRATES

No.	Nature	pH	Cationic Bonding Energies (cal/mole)		
			H	K	Ca
1	Ca Chloride	6.22	30	----	30
2	2.2% Ca-H Putnam	5.28	3367	----	3057
3	0.18% Ca-H Amb. XE64	5.72	3704	----	2605
4	0.90% Ca-H Wyo. Bent.	5.50	3268	----	2659
5	Ca Bicarbonate	6.45	2970	----	30
6	K Chloride	6.22	12	12	----
7	1.0% K-H Putnam	6.50	4562	1802	----
8	0.02% K-H Amb. XE64	6.28	2806	522	----
9	0.60% K-H Wyo. Bent.	6.60	4533	1636	----
10	K Bicarbonate	6.85	4550	12	----

to choose a definite value for the equivalence of the colloidal system. In the case of the chlorides, the values given in Table 2 are made up of a small addition corresponding to the activity coefficient in a solution of the given ionic strength. In the bicarbonate systems the value of  $c$  is the molar concentration, since each molecule of bicarbonate carries one hydrogen.

## EXPERIMENTAL PROCEDURES

### Preparation of Substrates

*Salts:* Stock solutions of potassium and calcium chlorides and of their mixtures were made up so that 50 ml. upon dilution to 19 liters, would give the required concentrations.

Potassium bicarbonate was weighed out and a stock solution prepared. Calcium bicarbonate solution was prepared by bubbling carbon dioxide through calcium carbonate suspensions. After excess calcium carbonate had settled out, the supernatant liquid was removed and the calcium content determined using a flame photometer. These solutions were then diluted as required.

Analyses were made of the substrates over the 20 day period of plant growth. The averages were used in calculating the figures given in Table 1. It will be noted that a number of deviations from the planned figures occurred.

*Putnam Clay:* The clay was taken from the subsoil of Putnam silt loam about three miles west of Centralia, Mo., on Highway 22. The lumps were soaked in distilled water and dispersion was effected by adding sodium hydroxide to pH 7.5, followed by vigorous stirring. Sand and silt were removed after sedimentation, and the clay fraction was heated at 90° C with hydrogen peroxide solution to oxidize organic matter. The system was diluted and passed through a Sharples supercentrifuge under such conditions of flow and rotational speed that only the fraction less than 0.2 $\mu$  in equivalent spherical diameter passed through. The soluble anions were removed from this suspension by passing it through a bed of an anion exchanger, Amberlite IR 4B in the free base form. This reduced the sulphate content from 12.5 p.p.m. to an undetectable amount, and the chloride from 25 p.p.m. to a trace.

This anion-free suspension containing chiefly calcium and sodium as the exchange cations was then converted in suitable quantity to the homionic Ca clay, K clay or H clay by passage through a large column of the sulphonic type cation exchanger Dowex 50 carrying the appropriate cation.

A special exchange column with reservoir and pump was constructed after a model successfully operated at the Shell Development Co., Houston, Tex. (designed and built there by D. R. Lewis and N. Dickerson). In the conversion to a Ca clay, 50 gm. of clay could be passed through the column before sodium began to appear. Using this exchanger appropriate amounts of Ca, K and H clays were prepared. These were mixed as soon as possible after preparation to

give clays with the required proportions of the three cations for use as substrates. A week was allowed for the attainment of equilibrium.

For the definition of  $K/\sqrt{Ca}$ , samples of the substrates were brought to equilibrium with dilute solutions of calcium and potassium chlorides such that little change in composition occurred. The clear supernatant liquids were analyzed for K and Ca by using the flame photometer.

*Wyoming Bentonite:* The clay sample used was from Clayspur, Wyo., and is largely a sodium montmorillonite. It was readily dispersed by stirring in water. Sand and silt particles were removed by sedimentation. The dilute suspension was concentrated using slow passage through a Sharples supercentrifuge. Some of the very finest clay, together with the bulk of the soluble electrolyte impurities were thus removed. The less than  $1\mu$  clay was diluted to about 1 percent and passed through a bed of Amberlite IR 4B to remove sulphate and chloride. The suspension was then converted from the Na form to the Ca, K or H form. These were mixed, diluted, and analyzed as described for Putnam clay.

*Amberlite XE 64:* The material, kindly furnished by Rohm and Haas, was ground dry in a porcelain ball mill, using agate balls, to produce a sufficient proportion of fine particles. The ground resin was suspended in water and particles which settled less than 10 cm in 8 hours were removed by decantation (equivalent spherical diameter less than  $6\mu$ ). The resin as received was chiefly in the H form, with some sodium present. The latter was removed by passage through the hydrogen exchange column described above. Potassium saturation was carried out by titration with potassium hydroxide solution. Another portion was converted to the calcium form by addition of pure calcium oxide, obtained by heating weighed amounts of the carbonate. The Ca, K and H resin suspensions were mixed and equilibrated for at least a week before use.

### Procedures in Growth of Plants

*Apparatus:* Plants were grown in Pyrex jars about 15 cm high and 10 cm in diameter (Figure 1). These were closed by corks, with holes for plant stems and the necessary tubing. Constant head siphons connected to five-gallon glass containers provided continuous flow of substrates. Aeration was effected by sintered glass bubblers connected to a source of compressed air.

*Nutrient Regulation:* To secure the most economical use of substrates, calculations of nutrient needs were based on Upchurch's experiments. It was assumed that nutrient requirements would be proportional to weight increase and that the usual exponential curve connecting weight with time would apply. From these data delivery schedules for the nutrients were set up. Substrates were changed manually up to three times daily. Continuous flow then provided for larger requirements.

*Germination and Growth:* Soybean seeds selected for uniformity were soaked two hours in distilled water, then placed between layers of damp filter

# Schematic Diagram of Plant Growth Apparatus

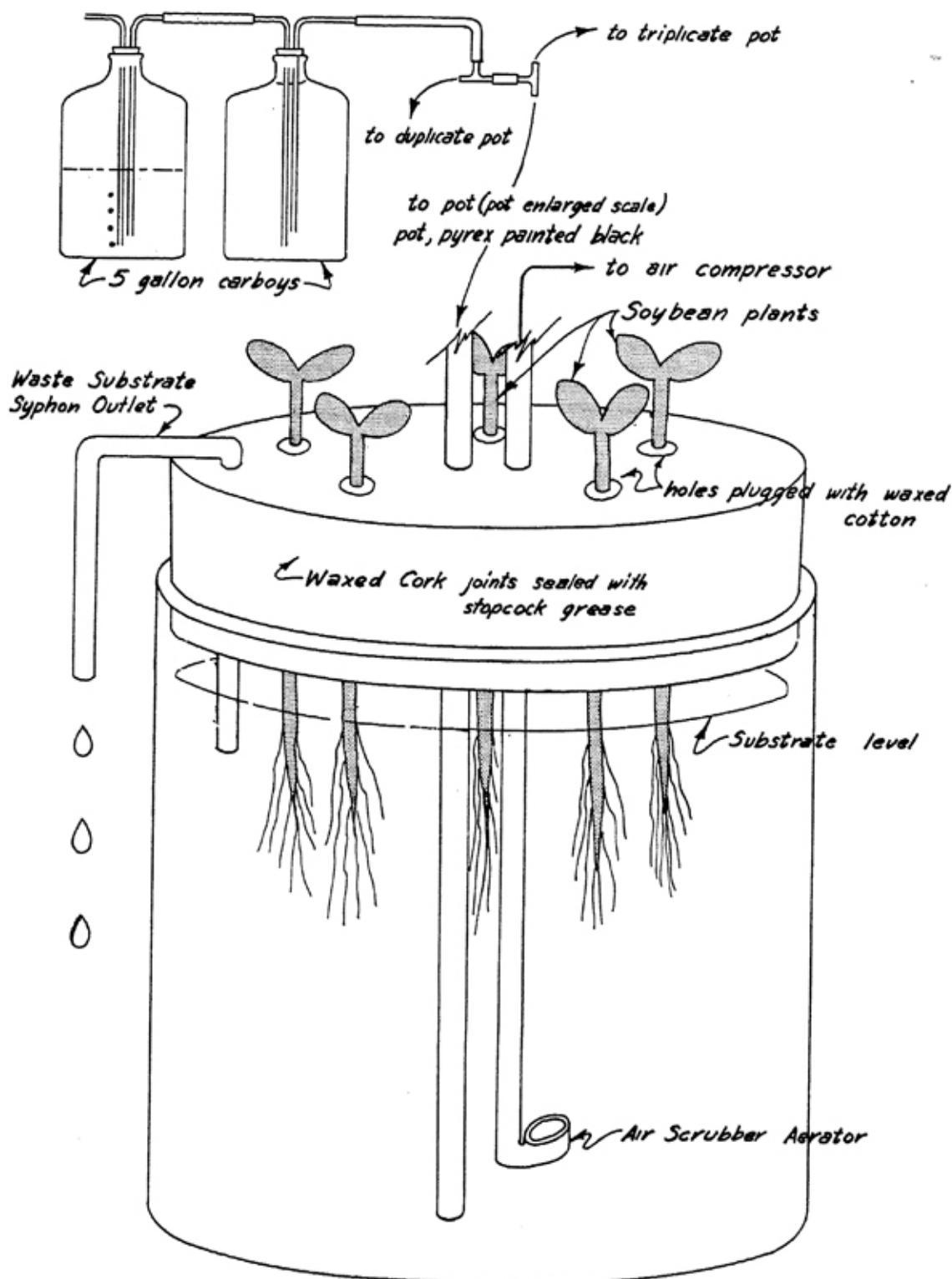


Figure 1

paper. In two days roots from 0.5 to 1.5 inches had sprouted. On the fourth day the seedlings were placed in each pot. Two further days were allowed for root growth, then on the sixth day the substrates were started. The opening cotyledons were green and the roots were from 1 to 3 inches long. On the seventh day true leaves began to appear. On the ninth day the pots were thinned to five plants. The second leaves began to show on the 11th day. Then the pots were sealed with silicone type stopcock grease, and wads of cotton coated with this were carefully tamped into the holes through which the stems passed. In this way the tops were effectively separated from the roots and substrates so that the upper parts could be sprayed several times daily with a nutrient solution designed to supply nitrate, sulphate, phosphate, ammonium, and magnesium. [26.4 grams diammonium hydrogen phosphate, 51.28 grams magnesium nitrate hexahydrate, and 26.64 grams magnesium sulphate were dissolved in water. Hydrochloric acid was added until white flocs of magnesium phosphate just dissolved (pH 4.6), and the mixture was made up to 2 liters. This solution was diluted 100 times for the actual spray. No trace elements were included as it was thought that the seeds would contain sufficient amounts for the 20 days growth.] On the 13th day the second leaves reached full development. Photographs were taken on the 16th, 21st and 26th day, the latter being the day of harvest, 20 days from starting the substrates.

The pots were arranged in a greenhouse with whitewashed top. The temperature reached 100° F on only one day. Supplemental illumination was supplied from tungsten lamps suspended about 4 feet above the benches. Measurements of plant height were made in the 12th and 20th days of substrate use.

*Harvesting and Root Exchange:* At the termination of the growth period, the tubing was removed and the corks with tops and roots were handled as units in the following manipulations. The roots were rinsed in distilled water to remove adhering substrate. They were then transferred to a water bath cooled to 4°-7° C to reduce the metabolism rate. At this temperature the root mass was treated with 25 ml of  $10^{-4}$  molar calcium chloride solution (pH 6.4) for about 30 seconds. It was hoped in this way to effect a small dilute exchange. The resulting solution was stored in a refrigerator until analyzed. The root mass was then rinsed in distilled water; excess water was removed by blotting; and the pH of the damp roots was measured by pressing against the electrodes of a Backman pH meter. These values were reproducible to 0.1 pH unit.

The root mass was then washed with four successive 50 ml quantities of cold N/10 lithium chloride solution. The solutions were combined and retained for analysis. The lithium saturated roots were then rinsed in distilled water and blotted dry. At this point the roots were cut from the stems and placed in tared beakers. Fresh weights were taken before drying to constant weight at 60° C. Most of the tops were dried at 60° C and weighed. In one group, however, fresh weights and dry weights were both determined. Because of their small size, the

plants in the "potassium only" series were dried and analyzed whole, without separation of the roots.

*Chemical Analyses:* In the case of roots, the whole of the dried mass was treated with 10 ml concentrated nitric acid and allowed to react for several hours. The amber solution was then brought to a boil and 2 ml perchloric acid was added. Boiling continued with additions of both acids as needed until the solution was colorless. The mixture was then evaporated down to 1 to 2 ml, diluted with water and filtered into a volumetric flask.

The dried tops were treated similarly except that these were ground to pass a 60 mesh screen and 0.250 gram samples of the ground material were taken for wet ashing.

Calcium determinations were made using the Perkin-Elmer flame photometer with corrections for interference by potassium and sodium. Potassium determinations were made similarly, after dilution in some cases to employ the most sensitive range.

Lithium determinations were made on the digested roots by the flame photometer as a means of estimating the cation exchange capacity.

In all cases each pot containing five plants was handled and analyzed separately. Each treatment comprised three pots. It was thus possible to calculate standard deviations for the analyses as well as for the crop yields.

## EXPERIMENTAL RESULTS

### *Qualitative Observations During the Growth Period*

It will be recalled that the substrate treatments were begun six days from the start of germination. The period without nutrients evidently was somewhat of a drain upon the minerals in the seed, since analyses of the sprouts showed extremely low calcium, and moderately low potassium. This manifested itself at early stage after the treatments began. In practically all cases the first true leaves, were stunted, darker green than normal at the base, grading into a yellow region midway. The top of the leaf turned brown and curled upward giving a boat shape very similar to that obtained by cupping both hands. In the case of plants grown on balanced media the leaves grew out of this effect. When the leaf was too badly stunted new buds were formed at the cotyledons and a secondary bud took over the growth of the top. The plants which overcame this effect most successfully were those whose substrates contained the highest proportions of calcium. We are therefore inclined to regard this cupping effect as a calcium deficiency symptom.

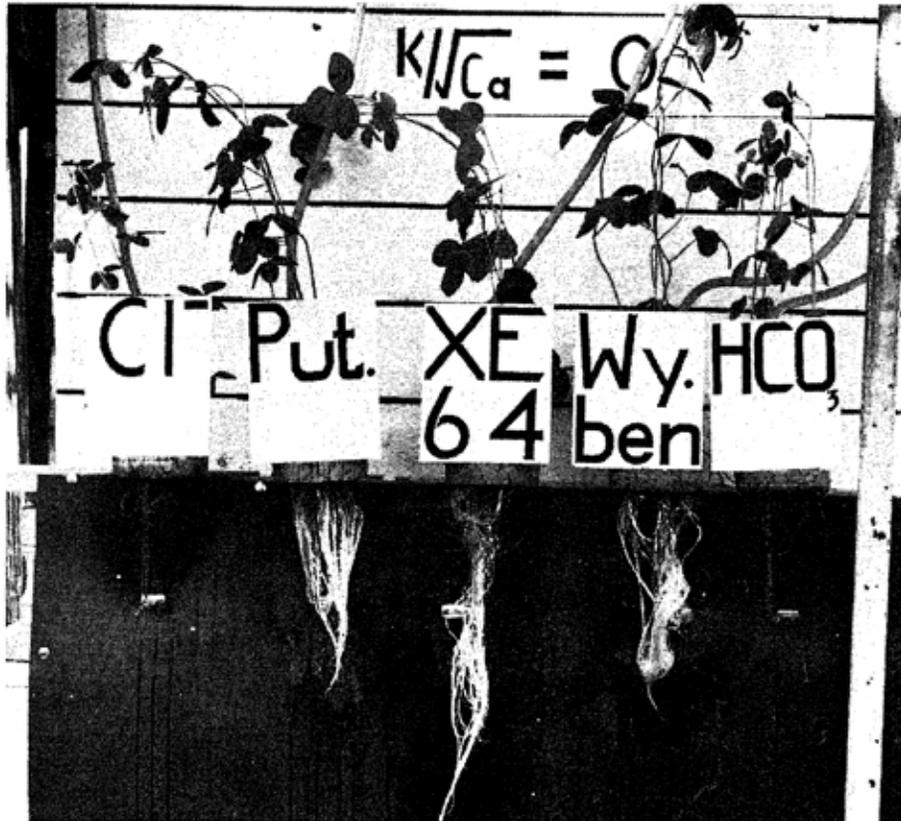
In the series which received no calcium, practically all the plants retrogressed between the 10th and 20th days, some being dead on the latter date. The plants of this series were weighed and analyzed whole, because of their small size. Root development was extremely poor (see Figure 2), as has often been noted for extreme cases of calcium deficiency.

Differences in root systems showed themselves at an early stage and became more accentuated as growth advanced. In general (except for the K only series) the roots grown on colloidal systems were white and succulent. Those grown on solutions were dark brown, fine and wiry. The two clay systems gave highly branched, bushy roots, while those grown on Amberlite XE 64 were elongated and much less branched.

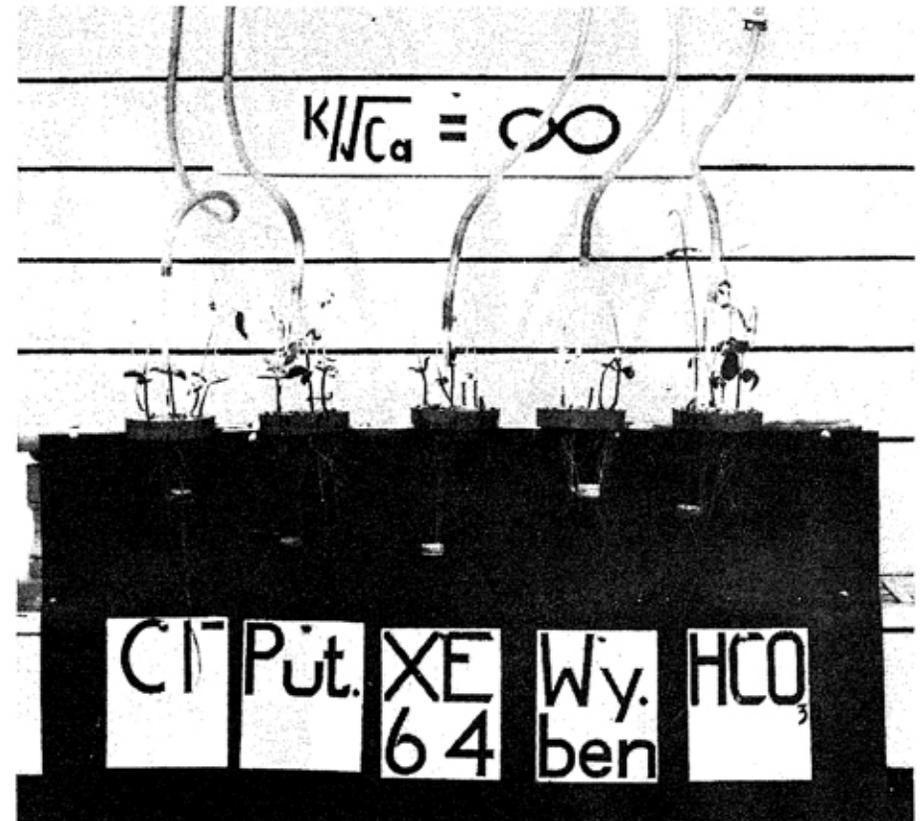
The visible characteristics of the roots showed little variation with potassium-calcium ratio or with the bonding energy of colloidal substrates for calcium. It can be seen from Figure 2 that increased concentrations of Ca and K in the solution substrates caused a definite increase in root growth.

Apart from the "cupping" effect and the other extreme manifestations of Ca deficiency in the K only series, no abnormalities were apparent in the tops until the 10th day, when slight iron deficiency symptoms showed themselves in all treatments except those on the Putnam clay substrate. It is not surprising that plants should be able to obtain adequate iron from this beidellitic clay, which contains 8 to 9 percent  $\text{Fe}_2\text{O}_3$ . Since the growth period was almost completed, no attempt was made to rectify the situation, and mild chlorosis continued thereafter to the 20th day.

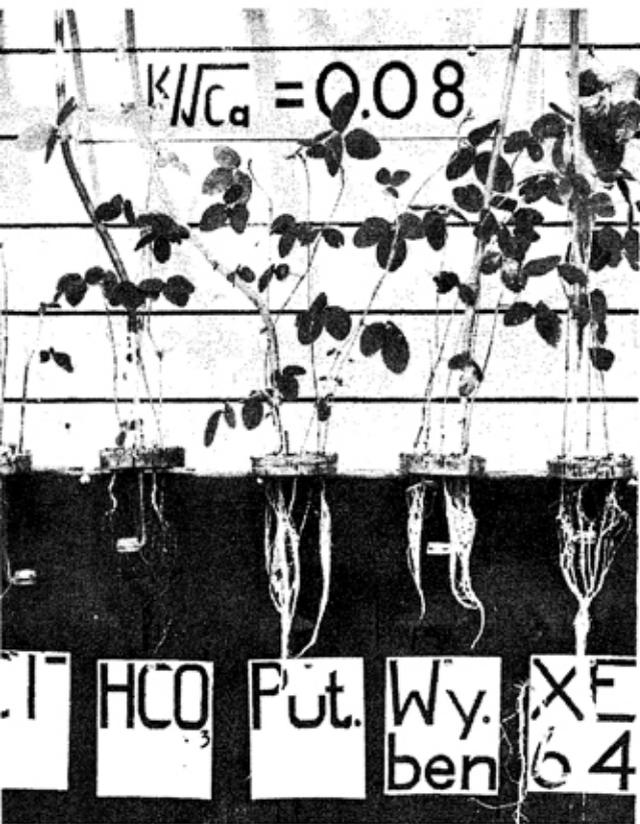
Figure 2  
Appearance of Plants on 20th Day, Immediately Before Harvest



a. Treatments 1-5.



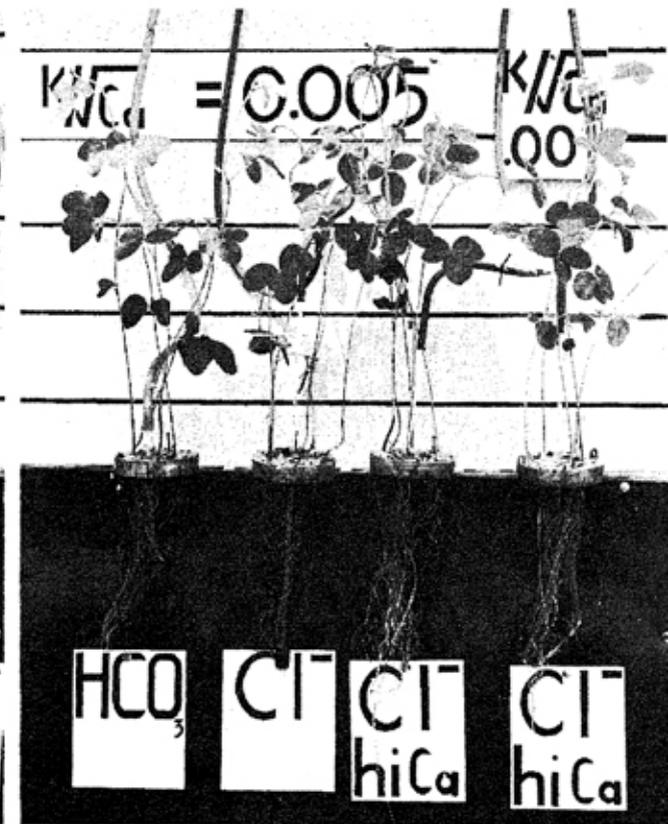
b. Treatments 6-10.



c. Treatments 11-15.



d. Treatments 16-20.



e. Treatments 21-24.

The early onset of severe calcium deficiency probably caused somewhat greater variability among plants of a given pot and among pots of a given treatment than would normally have been found. Plant heights were measured on the 12th and 20th days, and for the most part differences persisted. Figure 2 gives an idea of the relative growth of tops and roots immediately before harvest on the 20th day. The factors mentioned above are qualitatively apparent from these photographs. In each series the value of  $K/\sqrt{Ca}$  as originally designed is given. This serves only as a rough guide. The actual values achieved are found in Table 1.

### Cation Exchange Properties and Composition of Roots in Different Substrates

*Small Dilute Exchange:* The reaction with  $10^{-4}$  molar calcium chloride solution increased considerably the total concentration of the external solution. This has been observed previously in this laboratory for the first treatment of plant roots (6). Apparently, soluble constituents from the root pass into solution in addition to ions released purely by cation exchange. The equivalent cation concentration of the external solution increased from  $2 \times 10^{-4}$  to a maximum of about  $3 \times 10^{-4}$  in certain treatments. No great reproducibility was achieved between replicates.

The ratios of  $K/\sqrt{Ca}$  determined by this method differed considerably from those of the plant nutrient substrates (Table 3). For each type of substrate

TABLE 3--CATION EXCHANGE PROPERTIES OF SOYBEAN ROOTS

No.	Substrate	Root Exch. Cap. in m.eq./100gm.	$K/\sqrt{Ca}$		Root K/Ca molar ratio			
			Sub- strate	Small Exch.	Sub- strate	Small Exch.	Total Exch.	Total Content
1	Ca Chloride	4.9	----	.0028	----	----	----	----
11	K-Ca Chloride	6.6	.078	.006	11.5	0.60	----	4.1
16	K-Ca Chloride	7.8	.021	.003	3.0	0.28	----	3.1
22	K-Ca Chloride	11.3	.0065	.009	0.16	0.87	6.0	4.8
23	K-Ca Chloride	15.6	.0056	.013	0.044	1.29	5.9	10.6
5	Ca Bicarbonate	6.2	----	.008	----	----	----	----
12	K-Ca Bicarbonate	10.6	.057	.011	11.5	1.21	8.4	3.8
17	K-Ca Bicarbonate	9.3	.013	.005	3.0	0.55	5.1	2.3
21	K-Ca Bicarbonate	19.3	.0042	.012	0.16	1.23	1.8	5.4
2	Ca Putnam	11.0	----	.018	----	----	----	----
13	K-Ca Putnam	16.7	.042	.023	0.38	2.95	69	417
18	K-Ca Putnam	12.9	.011	.029	0.11	3.92	8.3	794
4	Ca Wyo. Bent.	11.5	----	.020	----	----	----	----
14	K-Ca Wyo. Bent.	13.8	.042	.020	0.22	2.62	5.4	163
19	K-Ca Wyo. Bent.	13.6	.010	.022	0.02	2.53	1.0	65
3	Ca Amb. XE64	14.0	----	.011	----	----	----	----
15	K-Ca Amb. XE64	24.6	.044	.023	0.33	3.58	8.3	5.9
20	K-Ca Amb. XE64	17.8	.011	.011	0.009	1.42	3.9	6.6

the ratio  $K/\sqrt{Ca}$  determined by the root exchange was less than the ratio of the original substrate for high values of the latter, but lower for low values. This indicated that for each substrate there should exist a certain nutrient ratio  $K/\sqrt{Ca}$  which gives an equal ratio by small dilute exchange applied to the roots. Donnan theory implies that all nutrient ratios should give correspondingly equal values by such exchange.

This lack of correspondence with the requirements of Donnan theory may be related to several causes. First, the root exchanges, though undoubtedly dilute, were not strictly small, since in some cases 30 percent of the exchangeable K plus Ca was removed. Second, the fact that soluble material passed from the roots to the solution may easily have affected the cation ratios. Third we have the general fact that during growth roots and substrates are not in a true equilibrium. Fourth, it is quite conceivable that the basic electrochemical properties of roots are dependent upon the cationic proportions and concentrations in the substrate.

From our limited and not highly precise determinations, it is not possible to assess the relative importance of these disturbing factors.

*Total Exchange:* The repeated exchange against 0.1 molar lithium chloride solution was designed to bring all exchangeable cations into solution. The analyses for K and Ca were made by the flame photometer and great variability was found in the values for individual pots of a single treatment.

The ratio  $K/Ca$  calculated from these results was discussed along with that for small dilute exchange, and that for total content under the two preceding subheadings.

Between the small dilute exchange and the total exchange experiments, the pH of the moist root mass was measured by pressing the roots against the glass-calomel electrode pair. The variations in these values among replicates of the same treatment were surprisingly small. All values lay in the range 5.5 to 5.9, and there were no marked variations as between treatments.

*Exchange Capacity of Roots:* From the total lithium content of roots which had been washed in distilled water and dried after the total exchange experiment, it was possible to calculate the cation exchange capacity, assuming that no lithium had been metabolized or had diffused into the interior of the roots during the exchange treatments and washings at 4 to 7° C. The values for individual pots of a given treatment showed large variations in a few cases, but for the most part the precision was higher than in the K and Ca determinations just described. The results are given in Table 3.

It will be noted that the colloidal substrates are associated with root systems having higher exchange capacities and a lighter color than the corresponding chloride and bicarbonate systems. The bicarbonate systems give higher values than the chloride systems. The actual capacities found are, on the average, somewhat low as compared with other determinations in the literature. The injury

caused by low calcium seems to show itself in low values for the root exchange capacity. Unfortunately the roots developed on the potassium only series were too small for measurement of the exchange properties.

*Potassium-Calcium Ratios of Roots.* It is interesting to compare the simple molar potassium/calcium ratios of the substrates with those of the root surfaces and of the total root composition (Table 3). In this tabulation the precision of the columns headed "Substrate" and "Roots total content" is much higher than those headed "small exchange" and "total exchange." The change in K/Ca ratio shows in general an increase in the order, substrate concentration, substrate activity, root small exchange, root total exchange, root total content, but there are a number of exceptions, the most notable being the chloride and bicarbonate systems 11, 12, 16, 17.

Note that in 16 and 17 the ratio K/Ca in the roots is not very different from that of the substrate. In 11 and 12 it is distinctly less, whereas in 21, 22 and 24 it is much greater.

The three colloidal substrates show very great differences in K/Ca ratios for the roots, which in both series fall from very high values for Putnam clay

TABLE 4--CALCIUM DATA FOR VARIOUS TREATMENTS

Number	Substrate	K/ $\sqrt{\text{Ca}}$	TOPS		TOPS & ROOTS
			Moles Ca per gm x 10 <sup>-5</sup>	Yield gm per pot	Total Ca Moles x 10 <sup>-5</sup>
Sprouts	Dist. Water		3.25	0.732	2.38
1	Ca Chloride		0.40	0.808	1.02
2	Ca Putnam		9.27	1.394	13.9
3	Ca Amb. XE64		16.6	1.368	31.8
4	Ca Wyo. Bent.		8.16	1.304	11.7
5	Ca Bicarbonate		1.20	1.055	2.43
6	K Chloride	Plants harvested	} -----	0.797	-----
7	K Putnam	whole. Ca too small		0.825	-----
8	K Amb. XE64	for quantitative de-		0.785	-----
9	K Wyo. Bent.	termination.		0.835	-----
10	K Bicarbonate			0.804	-----
11	K-Ca Chloride	.078	0.63	0.911	1.28
12	K-Ca Bicarbonate	.057	1.96	1.175	3.45
13	K-Ca Putnam	.042	10.3	1.585	16.4
14	K-Ca Wyo. Bent.	.042	2.6	1.105	3.01
15	K-Ca Amb. XE64	.044	13.0	1.599	24.5
16	K-Ca Chloride	.021	2.2	0.718	2.33
17	K-Ca Bicarbonate	.013	3.5	0.974	4.70
18	K-Ca Putnam	.011	8.4	1.896	16.0
19	K-Ca Wyo. Bent.	.010	2.33	1.249	3.19
20	K-Ca Amb. XE64	.011	16.1	1.665	35.6
21	K-Ca Bicarbonate	.0042	15.1	1.491	24.0
22	K-Ca Chloride	.0065	8.8	1.447	14.6
23	K-Ca Chloride	.0056	18.5	1.500	29.1
24	K-Ca Chloride	.0016	20.5	1.467	30.0

through moderate values for bentonite to low values for Amberlite IR XE 64. The actual Ca contents of the roots after removal of the exchangeable Ca by lithium chloride solutions were very low for the two clays, but relatively high for the Amberlite. The potassium contents varied relatively little. Reference to data for tops (Tables 4 and 5) indicates that these extremely high values for K/Ca in roots are not characteristic of the whole plant. Apparently in succulent healthy roots, passage from the exchange sites to the tops is extremely efficient and little non-exchangeable calcium may remain in the roots themselves. Thus a relatively small difference in the bonding energy relationships of roots and colloid for single cations may show itself in an exaggerated way in the root composition when the exchange cations have already been removed.

### *Plant Composition in Relation to Treatment*

*Calcium Content:* Table 4 gives the summarized figures for calcium in the tops, yield of tops and total calcium found in the tops plus roots per pot. Great variations in calcium content were achieved, together with significant variations in yield.

TABLE 5--POTASSIUM DATA FOR VARIOUS TREATMENTS

Number	Substrate	K/ $\sqrt{\text{Ca}}$	TOPS		TOPS & ROOTS
			Moles K/gm $\times 10^{-5}$	Yield in gm	K Moles $\times 10^{-5}$
Sprouts	Dist. Water	(whole plants)	31.5	0.732	23.0
1	Ca Chloride		36.3	0.808	32.0
2	Ca Putnam		26.4	1.394	40.7
3	Ca XE64		34.6	1.368	58.7
4	Ca Wyo. Bent.		29.1	1.304	41.4
5	Ca Bicarbonate		29.6	1.055	32.3
6	K Chloride	whole plants	54.3 70.8 59.3 68.7 53.2	0.797	43.3
7	K Putnam			0.825	58.3
8	K XE64			0.785	46.8
9	K Wyo. Bent.			0.835	57.8
10	K Bicarbonate			0.804	43.0
11	K-Ca Chloride	.078	45.1	0.911	43.8
12	K-Ca Bicarbonate	.057	48.0	1.175	70.3
13	K-Ca Putnam	.042	58.5	1.585	109.0
14	K-Ca Wyo. Bent.	.042	61.6	1.105	79.7
15	K-Ca XE64	.044	86.1	1.599	155.9
16	K-Ca Chloride	.021	41.1	0.718	35.3
17	K-Ca Bicarbonate	.013	34.3	0.974	36.8
18	K-Ca Putnam	.011	51.7	1.896	112.8
19	K-Ca Wyo. Bent.	.010	60.1	1.249	87.8
20	K-Ca XE64	.011	68.6	1.665	130.3
21	K-Ca Bicarbonate	.0042	48.3	1.491	81.1
22	K-Ca Chloride	.0065	41.6	1.447	68.2
23	K-Ca Chloride	.0056	44.9	1.500	78.9
24	K-Ca Chloride	.0016	24.7	1.467	46.6

This table affords clear evidence that absence of calcium during germination caused injury which could be overcome by the use of substrates of high Ca/K ratio or the operation of other factors favoring Ca uptake. Considering first the chloride solutions, we find that the total Ca of the plants was less than that of the sprouts in the potassium chloride and calcium chloride substrates (6 and 1) and in the mixed system at  $K/\sqrt{Ca} = 0.78$ . (no. 11). The two were almost equal for the mixed chloride system at  $K/\sqrt{Ca} = 0.021$  (no 16). A large increase in Ca accompanied the reduction in  $K/\sqrt{Ca}$  ratio to 0.0065 and the simultaneous tenfold increase in Ca activity (no. 22). A further increase in Ca and K activities at almost this same  $K/\sqrt{Ca}$  ratio caused a marked increase in Ca (no. 23). Reduction in the  $K/\sqrt{Ca}$  ratio to 0.0016 at practically constant Ca activity (no. 24) produced a plant whose calcium content was not significantly different from that of substrate 23. These results clearly reflect the strong influence of the absolute level of Ca activity and the subordinate importance of the  $K/\sqrt{Ca}$  ratio.

In bicarbonate systems only the K substrate (no. 10) gave a Ca content below that of the sprouts. The calcium bicarbonate substrate (no. 5) showed equality. In the mixed systems, we have an interesting series in which (no. 12) at a  $K/\sqrt{Ca}$  of 0.057 is exceeded by (no. 17) at  $K/\sqrt{Ca}$  of 0.013. Evidently some potassium is needed before Ca uptake can function normally; but a little suffices. Increase in Ca activity by a factor of 10 with simultaneous decrease of the  $K/\sqrt{Ca}$  ratio to 0.0042 produced a large increase in Ca content (no. 21). Here again we note the dominant effect of Ca activity, the ratio factor being subordinate.

All comparisons of bicarbonate systems with corresponding chloride systems show the former to give higher Ca contents by a factor of 2-3. This reflects the importance of the free energy change in the reaction between root and substrate. In bicarbonate systems, the exchangeable hydrogen released from the root produces slightly dissociated carbonic acid instead of hydrochloric acid. Expressed in calories per mole this difference is of the order of 3000 to 4000 calories but if stronger organic acids than carbonic participate then the difference would be smaller.

We have three groups of colloidal systems to consider; 2, 3, 4 receiving Ca only; 13, 14, 15, at  $K/\sqrt{Ca}$  about 0.04; and 18, 19, 20 at  $K/\sqrt{Ca}$  about 0.01. All three sets had calcium ion activities around  $4 \times 10^{-5}$  moles/liter, although in the mixed systems there may be deviations of unknown amount from this value.

Considering first the three Wyoming Bentonite systems, (no. 4) Ca only, (no. 14) at  $K/\sqrt{Ca}$  0.04, and (no. 19) at  $K/\sqrt{Ca}$  .01. In absence of potassium (no. 4), calcium uptake was greatly superior to that in the corresponding bicarbonate system (no. 5). However, in presence of potassium, (nos. 14 and 19), calcium uptake and yields were similar to those of bicarbonate systems (nos. 12 and 17) and much lower than in the other two colloidal substrates (nos. 13, 15, 18, 20.) In previous comparisons of Ca bicarbonate and Ca Wyoming bentonite

systems, McLean found the latter to be slightly superior in Ca removal, whereas Upchurch found the bicarbonate substrate to be somewhat more effective. No good explanation for our present results can be offered.

Putnam clay, in all three cases (nos. 2, 13, 18), is much superior to the corresponding bicarbonates (nos. 4, 12, 17); but XE64 gives even higher calcium contents (nos. 3, 15, 20). Any superiority which Putnam clay might possess in terms of possible provision of trace elements (e.g. iron) could not be shared by the synthetic resin XE64, yet the latter is a remarkably effective substrate. Thus toward plants, as in its chemistry, it resembles soil organic matter.

*Potassium Content:* Table 3 gives data for potassium in the tops, yields of tops, and total potassium per pot removed by the whole plants. Variations with treatment were much smaller for potassium than for calcium. In part this may be due to unforeseen contamination which is clearly apparent in treatments 1 to 5. Here potassium removal considerably exceeded that found in the sprouts, yet all were supposedly calcium only treatments. The exchange resin XE64 apparently contained considerable amounts of available potassium. The consequence was that plants grew well; they accumulated moderate amounts of potassium and were thereby enabled also to accumulate quite large amounts of calcium. Thus in the series 1 to 5 we have both potassium and calcium as variables.

In the potassium only series, nos. 6 to 10, which showed early signs of extensive calcium deficiency, the potassium contents were not unduly high. Lack of calcium showed itself in a severe limitation of growth and also in the absence of luxury levels of potassium accumulation.

In the whole series only one treatment, No. 15, in which XE64 was used at a  $K/\sqrt{Ca}$  ratio of 0.044, gave a potassium content over 3 percent. In McLean's experiments (9) all the calcium-potassium systems gave K percentages over 3 and in Upchurch's comparisons two cases were slightly below 3 and two above. The cause of this generally lower K content appears largely to be connected with low Ca content. In treatments 11 (chloride) and 12 (bicarbonate) at a  $K/\sqrt{Ca}$  ratio around 0.07, the relatively high K activity of  $5 \times 10^{-4}$  did not cause notable potassium accumulation, apparently because the Ca contents were extremely low. At the other end of the scale, when Ca greatly exceeded K in the substrate, as in treatment 24 (with a Ca activity of  $40 \times 10^{-4}$  and a K activity of  $0.6 \times 10^{-4}$ ), a distinct but not extreme depression of the potassium content occurred. However, at this very low  $K/\sqrt{Ca}$  ratio of 0.0016, good yields of tops were obtained, almost identical with those at 0.006.

Comparison of the two mixed chloride systems 22 and 23 at almost the same  $K/\sqrt{Ca}$  ratio of 0.006 indicates that increase in the absolute concentration level of both ions has much less influence on the potassium than on the calcium.

Comparable chloride and bicarbonate systems (nos. 11, 12, 16, 17, 22, 21) show much smaller variations in K content than in Ca, irrespective of the

K/ $\sqrt{\text{Ca}}$  ratio. Thus a number of comparisons lead to the conclusion that when young soybeans are recovering from a state of pronounced calcium deficiency, adequate potassium nutrition is only achieved with the aid of additional calcium. In this situation other factors such as the free energy change in the hydrogen-potassium exchange from root to medium cease to show themselves in the potassium figures, although clearly evident for calcium.

The three colloidal media all gave plants with higher potassium contents than the corresponding chloride or bicarbonate systems. The order was Putnam < Bentonite < XE64 in both series. However, the bentonite systems gave plants with low calcium contents and distinctly lower yields than those grown on Putnam clay or Amberlite XE64. Hence the total potassium and the total calcium uptakes both followed the order Bentonite < Putnam clay < Amberlite XE64. The superiority of the Amberlite XE64, as shown in mixed K-Ca systems, may well be caused by its relatively low bonding energy for potassium. In the potassium only series this in turn was probably overshadowed by possible release of small amounts of magnesium and calcium from Putnam clay and Bentonite, which facilitated potassium uptake.

As regards total uptake of potassium, Putnam clay gave similar values at the K/ $\sqrt{\text{Ca}}$  ratios of 0.042 and 0.011. Wyoming Bentonite was slightly more effective of 0.010 than at 0.042. Amberlite XE64 was somewhat more effective at 0.044 than at 0.011.

The mixed K-Ca chloride and bicarbonate systems make a very interesting series as regards potassium. In passing from 11 and 12 at K/ $\sqrt{\text{Ca}} \cong .06$  to 16 and 17 at K/ $\sqrt{\text{Ca}} \cong .02$  the calcium activity was held constant and the potassium activity decreased. Correspondingly the total potassium uptake decreased. In comparing 16 and 17 with 22 and 21 at K/ $\sqrt{\text{Ca}} \cong .005$  the potassium activity remains constant while the calcium activity increases. However 21 and 22 show a noteworthy increase in potassium uptake, evidently related to the improved calcium nutrition, as shown also in calcium content and in greatly increased yields of dry matter.

It is very clear from all these comparisons that in recovery from calcium deficiency the most important factor is the absolute calcium activity of the substrate. The K/ $\sqrt{\text{Ca}}$  ratio is almost completely obscured in its effects and the free energy of the cation exchange reaction between plant root and nutrient medium shows itself in a few cases, such as the bicarbonate-chloride comparisons. If accurate K and Ca activities had been available for the mixed colloidal substances this factor would have been more completely assessed.

The somewhat confusing results obtained in this interpretation may be compared with those obtained by Bartlett and McLean (2) in a recent study of potassium and calcium uptake from clay and solution substrates using soybeans, barley and buckwheat as the experimental plants. Potassium uptake was found to be more closely related to the total amount present in the substrate than to its activity. With soybeans, uptake of calcium was related to the Ca activity,

while in barley and buckwheat Ca uptake was greater from solutions than from clay suspensions of the same Ca activity. No consistent relationship between growth or uptake of K or Ca and the  $K/\sqrt{Ca}$  ratio of the substrate can be traced in these experiments. Thus, although the details differ somewhat from those of our own experiments, since Bartlett and McLean reported no visible evidence of calcium deficiency, there is considerable similarity in the results, especially in the lack of control shown through the  $K/\sqrt{Ca}$  ratios.

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