provide evidence that the physiological role of boron is likely related in part to the complexing property of the borate ion. On the basis of translocation studies utilizing C-14 sucrose and tests on the effect of sugar and citric acid applications to boron-deficient plants, it does not appear that boron is directly functional in sugar translocation because of the formation of a sugar-borate complex.

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THE EFFECT OF CALCIUM ON POTASSIUM ACCUMULATION IN CORN AND SOYBEAN ROOTS[1]

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It has often been observed that different species of plants will accumulate markedly different amounts of nutrient cations from the same root medium (3). This is particularly striking in the case of mono- and divalent cation absorption by grasses and legumes (5, 8); the former tend to accumulate much more potassium and less calcium than the latter.

Such differential absorption appears to have ecological and agronomic importance when grass and legumes species are members of the same plant society, as in mixed pastures. The productiveness of legumes in such pastures tends to fall sharply after a few years, a process which can be slowed down, but not stopped, by liberal applications of potassium fertilizer (1). Apparently, the potassium accumulating mechanism of legumes cannot compete successfully with that of grasses unless the concentration of the ion is quite high.

The amount of K that a plant absorbs, however, will depend not only on the species and the concentration of the ion in the root medium, but also on the relative amounts of Ca and Mg present. It has been demonstrated that the total amount of cations absorbed by a plant from differing media tends to approach a constant, but that the relative accumulation of K, Ca, and Mg is dependent upon the ratios of these ions in the nutrient medium (4, 14). High concentrations of K will tend to depress the accumulation of divalent cations, and vice versa. Olsen (11) has demonstrated that different species respond differently to changing Ca/K ratios, but the species tested did not include legumes.

A complicating factor of unknown significance in the potassium accumulation of plants is the promoting effect of Ca, and to a lesser degree of Mg and other di- and trivalent ions, on the uptake of K and associated anions (the Viets effect) (16). Studies of this effect have been made with barley (12) and Mung bean roots (15). However, no direct comparison of

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the magnitude of this effect in grass and legume species has been made and no assessment as to the importance of this effect in the relative cation composition of the two families is possible.

The experiments described here were performed in order to make such a comparison and to evaluate the effect of Ca on K uptake in terms of the kinetic treatment of Epstein and Hagen (6).

MATERIALS AND METHODS

Hybrid corn (WF 9 × M 14) and soybeans (Hawkeye) were used as representative grass and legume species. A preliminary nutrient culture experiment was run in the greenhouse to make certain these plants were characteristic of their families with respect to K and Ca accumulation.

Five-day-old seedlings were fixed with cotton plugs in a masonite lid, 2 corn and 2 soybean seedlings per lid, and the roots suspended in 7 liters of aerated, modified Hoagland solution in a glazed earthenware crock. By varying the relative amounts of $Ca(NO_3)_2$ and KNO_3 used, the Ca/K ratios indicated in figure 1 were obtained, all other ions remaining constant. The pH varied from 5.6 to 6.1; solutions were changed every 6 days. The experiments were done with full and with 1/5 strength nutrient solutions.

The plants were harvested after 4 weeks, weighed, dried, reweighed and ground in a Wiley mill. Samples were wet-ashed with nitric and perchloric acids for analysis. K and Ca were determined in a Perkin-Elmer flame photometer, Ca + Mg with Versenate (2) and Mg calculated by difference. The Ca standard solutions contained the average amount of K and Mg found in the samples as interfering ion.

Excised root experiments were done with the entire primary root from 4-day-old seedlings. The seeds were germinated at 28° C in Pyrex baking dishes on a 0.5-inch (1.25-cm) bed of washed quartz gravel covered with paper toweling and wetted with 10^{-4} M $CaCl_2$. The humidity in the germinator was maintained near saturation. Growth was vigorous, and root hairs and secondary roots were present.

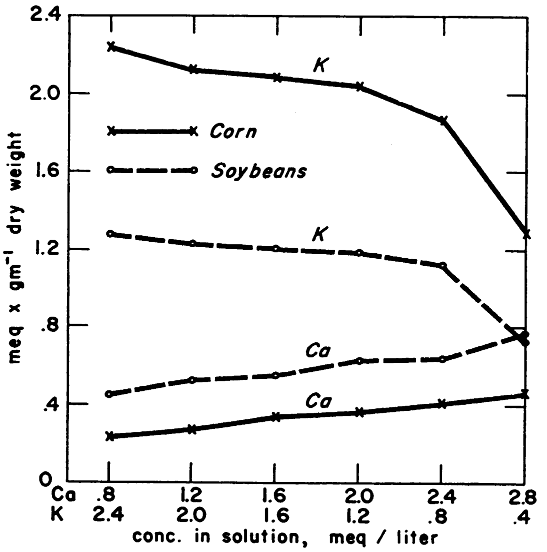
TABLE I

Rb^{86}/K UPTAKE BY EXCISED CORN ROOTS FROM K SOLUTIONS LABELED WITH TRACER Rb^{86}

HRS	K, MEQ/L	Ca, MEQ/L	Rb^{86}/K
	K by conductivity change *		
2nd	1	...	0.97
3rd	1	...	0.97
4th	1	...	0.97
5th	1	...	0.93
	K by flame photometer **		
0–1.5	0.8	...	1.01
0–1.5	0.8	0.5	0.91
0–1.5	1.6	...	1.02
0–1.5	1.6	0.5	1.07
0–3	0.8	...	1.00
0–3	0.8	0.5	0.98
0–3	1.6	...	0.99
0–3	1.6	0.5	0.95

* Uptake measured by loss from 1 meq/l KCl.

** Uptake measured by loss from solution with cation concentration as indicated, and equimolar Cl and PO_4 as anions (pH 5.6).



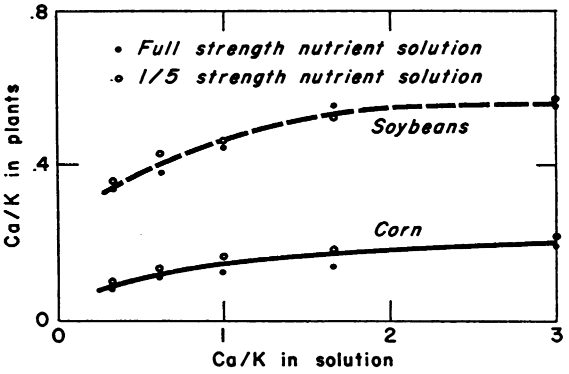


FIG. 1 (*above*). Ca and K composition of shoots of corn and soybeans grown together in nutrient solutions of varying Ca/K ratio for 4 wks.

FIG. 2 (*below*). Variation in the Ca/K ratio in the shoots of corn and soybeans as a function of Ca/K ratio in the nutrient solution.

Four entire primary roots were cut into approximately 1-inch (2.5-cm) pieces and placed in a 125-ml Erlenmeyer flask with 25 ml of solution; each treatment was replicated three or four times. Potassium solutions were labelled with Rb^{86}, and equimolar concentrations of Cl and PO_4 were present as anion; pH varied from 5.5 to 5.8. Solutions contained 1 % sucrose as it was found to decrease variability between replicate flasks.

The use of Rb^{86} as a tracer for K, as suggested by Roberts et al (13), is believed to introduce no appreciable error. To verify this, experiments were conducted in which, after a period of equilibration to allow for completion of exchange, the loss of Rb^{68} from solution was compared with the loss of K and with the decrease in conductivity (table I).

Flask containing the roots were placed on a unidirectional shaker, shaking at a rate of 100 cycles/min. Temperature control was maintained by having the flasks immersed to solution level in constant temperature water baths. At the end of 3 hours solutions were removed and roots rinsed once with deionized water. Exchangeable Rb^{86} was removed by adding 50 ml of solutions identical with those of the absorption period except for the absence of Rb^{86}, and keeping the flasks for 1 hour in an ice bath.

Roots from each flask were placed in Coors #1 china capsules, digested with HNO_3, and radioactivity determined directly from the capsules. Conversion factors for calculation of ion absorption were obtained from the radioactivity of the initial solutions.

RESULTS

The nutrient culture experiments show that corn and soybeans exhibit cation accumulation characteristic of their families, in that corn plants accumulate more K and less Ca than soybeans growing in the same solution (fig 1). Altering the Ca/K ratio of the solutions produced no significant difference in plant growth during a 4-week period or in the Mg content of either species and these values are not reported. If the Ca/K ratio of the solution is plotted against the Ca/K ratio in the plant, it is made apparent in the change in slope of the curves that relative cation accumulation in soybeans is somewhat more sensitive to changes in the composition of the solution (fig 2). This figure also shows that the Ca/K ratio in the plants is not much altered by a five-fold change in nutrient solution concentration.

The effect of increasing Ca concentration on K accumulation by the excised primary roots is shown in figure 3. The corn roots exhibit a typical Viets effect, in that Ca has increased K accumulation at this K concentration (1 meq/l); however, even minute amounts of Ca depressed K accumulation by soybean roots.

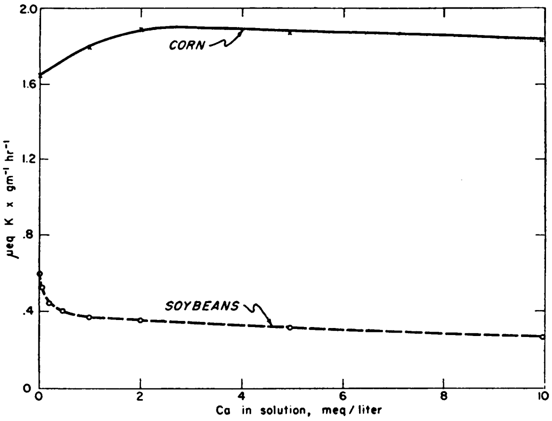


FIG. 3. The effect of increasing amounts of Ca on K accumulation by excised corn and soybean roots from 0.001 M KCl.

TABLE II

THE EFFECT OF TEMPERATURE CHANGES ON K_s AND V_m OF K ACCUMULATION IN CORN AND SOYBEAN ROOTS

PLANT	Ca, 0.5 MEQ/L	TEMPERATURES, °C		K_s		V_m	
		LOW	HIGH	LOW T	HIGH T	LOW T	HIGH T
Corn	−	20	26	0.42	0.39	1.09	2.10
Corn	+	20	28	0.19	0.19	0.77	2.11
Corn	+	23	31	0.19	0.20	1.46	1.99
Soybean	+	25	27	0.45	0.45	1.18	1.30

In order to study the kinetics of accumulation, the experiments were carried out in K concentrations between 0.4 and 2.0 meq/l, with or without 0.5 meq/l Ca. The results were then evaluated by the method proposed by Hanes (9), plotting S/V vs S, where S = K concentration in solution in meq/l and V = K accumulation in $\mu eq \times hr^{-1} \times gm^{-1}$ fresh weight roots. $V_m = 1/\text{slope}$, $K_s = V_m \times$ ordinate intercept. The regression lines were calculated by least squares.

The results of these experiments (figs 4, 5) show that the K_s for K accumulation is higher in soybeans than in corn, while V_m is lower. The effect of Ca is to lower K_s and V_m in both species. Mg has a similar but smaller effect (fig 6). The small values for K_s represent a greater affinity between the K ion and the postulated carrier.

If the four regression lines of figures 4 and 5 are plotted as V vs S (fig 7), typical curves illustrating the promoting influence of Ca are obtained. Overstreet et al (12) have reported similar curves for barley roots. The promotion of K accumulation by Ca at low K concentrations follows directly from the greater affinity between the ion and the carrier. Inasmuch as K_s is lowered by Ca proportionally less in soybean than in corn roots, with proportionally greater effect on V_m, the Viets effect in soybean roots is smaller and is limited to very low K concentrations. For this reason it was not detected in earlier experiments (fig 3).

The values for K_s and V_m were determined at different temperatures (table II). While V_m had a large temperature dependence, K_s remained essentially constant.

DISCUSSION

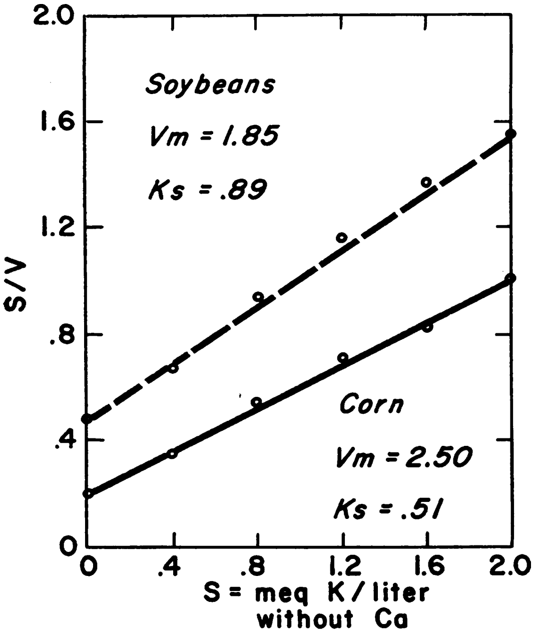
The kinetic treatment of ion accumulation introduced by Epstein and Hagan is based on the principles and assumptions of classical enzyme kinetics. The reaction for an ion M and an ion carrier R, which is metabolically changed to R′ while releasing M as an accumulated ion, is pictured thus:

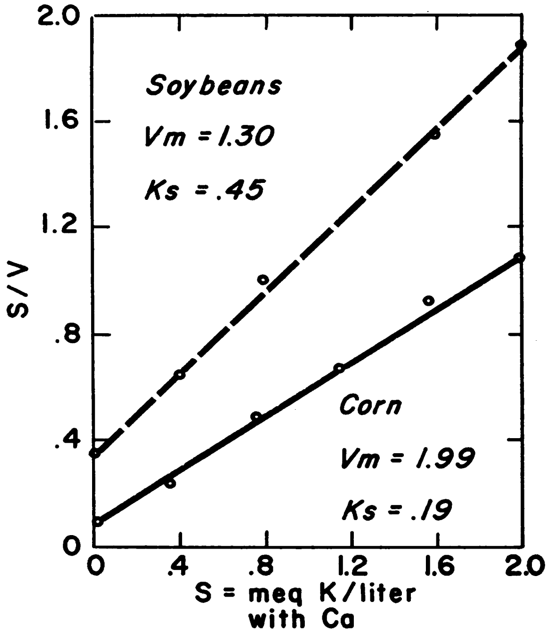
$$M_{\text{outside}} + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MR \xrightarrow{k_3} M_{\text{inside}} + R'$$

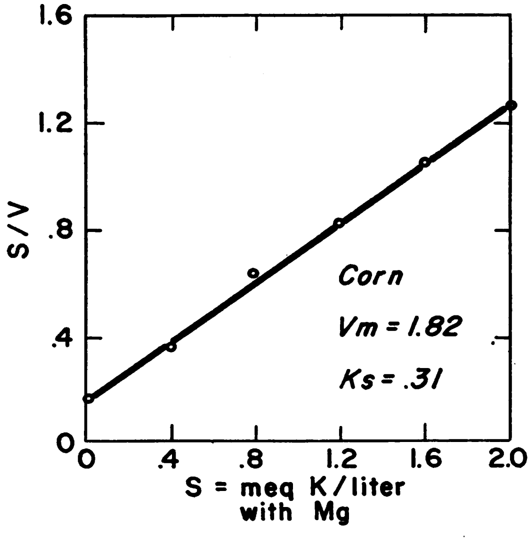
At a steady state of ion accumulation the concentration of MR will be constant, and it can be shown that the Michaelis constant,

$$K_s = \frac{k_2 + k_3}{k_1}$$

An assumption in these reactions is that k_3 is small as compared to k_2; that is, the reaction $MR \rightarrow M + R'$ is the rate-limiting reaction, depending upon metabolic energy and essentially irreversible due to large







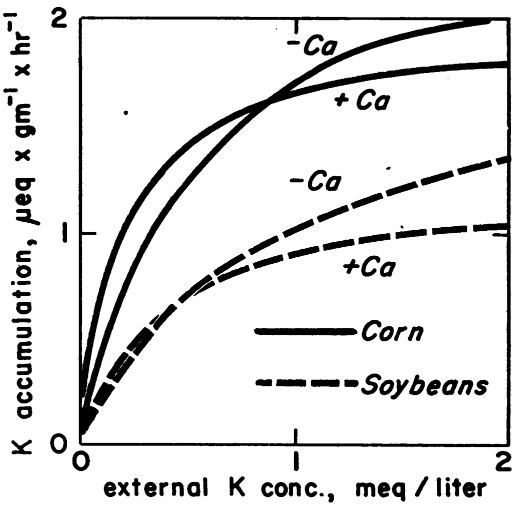


FIG. 4 (*upper, left*). Graphic kinetic analysis of K accumulation by excised corn and soybean roots in the absence of Ca. S = solution concentration; V = rate of K accumulation as $\mu eq \times gm^{-1} \times hr^{-1}$; V_m = maximum rate; K_s = Michaelis constant as meq/liter. Values at S = 0 are calculated intercepts.

FIG. 5 (*upper, right*). Same as figure 4 but in the presence of 0.5 meq Ca/liter.

FIG. 6 (*lower, left*). Same as in figure 4, for corn roots only, in the presence of 0.5 meq mg/liter.

FIG. 7 (*lower, right*). Rate of K accumulation by excised corn and soybean roots as a function of external K concentration. Curves derived from regression lines of figures 4 and 5.

negative free energy change. With this assumption, that $k_3 \ll k_2$, K_s approaches the true equilibrium constant for $M + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MR$. Evidence for the validity of this assumption can be found in table II, where K_s proves to be constant despite large changes in V_m induced by temperature variations. If k_3, which will change with changes in metabolic rate, were relatively large, K_s should have a large temperature dependence. Since it has not, $K_s \approx \frac{k_2}{k_1}$ and is approaching a true dissociation constant, independent of the metabolic factors which influence V_m.

With this assumption, that K_s closely represents the true dissociation constant of the ion-carrier complex, the effect of Ca on K accumulation seems to be two-fold. First, Ca increases the affinity between the K ion and its carrier (lower K_s) and second, it lowers V_m by a reaction which is independent of the reaction affecting K_s. Calcium could conceivably lower V_m by lowering the concentration of R, or by lowering the rate of carrier turnover, k_3. The kinetic treatment in itself does not distinguish between these possibilities.

Whatever the mechanism, it should be recalled that the effect of Ca in promoting ion uptake is not specific for K but has been observed for bromine and phosphate as well (10, 16); in addition Ca can be substituted to some degree by Mg and other di- and polyvalent ions (7, 16). It appears that divalent or polyvalent ions must generally serve to increase the affinity between some ions and their carriers, and at the same time inhibit some phase of carrier metabolism in a manner which is not competitive.

At moderate levels of K concentration, the presence of Ca will promote K uptake in corn and inhibit it in soybeans (fig 3). This phenomenon will partially explain the relatively high K/Ca accumulation ratios in corn as compared to soybeans; however, it should be noted that even in the absence of Ca, soybeans are less efficient in K accumulation due to a higher K_s and lower V_m.

SUMMARY

A study has been made of the kinetics of K accumulation by corn and soybean roots as affected by Ca. For both kinds of roots, Ca increases the affinity between the K ion and the postulated carrier; in a second reaction, independent of the first, Ca decreases the velocity of the metabolic phase of K uptake. The first effect of Ca is proportionally greater in corn, while the second is proportionally greater in soybeans. The net result of the two effects is to reduce maximum K accumulation in the presence of Ca more in soybeans than in corn.

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