BIOCHEMISTRY OF SALT ABSORPTION AND ACCUMULATION BY PLANTS WITH SPECIAL REFERENCE TO THE ROLE OF ETHER SOLUBLE ORGANIC ACIDS

By

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INTRODUCTION

Factors that influence salt absorption by plants have long been a subject of study. Only recently however, have the effects of certain metabolic processes in the plant been recognized as intimately connected with the process of salt absorption.

This investigation has been concerned with the cationanion balance in several plants with special reference to the role of ether soluble organic acids. A study has been make of the selective capacity of various plants with respect to inorganic ions; of the quantity and kinds of organic acids in a number of different crop plants grown in a uniform culture medium; of the correlation between inorganic cations and ether soluble organic acids in the different plants as a group, and of the preferential absorption of cations as influenced by specific organic acids. The relative importance of phosphate and organic acids as contributors to the titratable acidity of plant sap has also been considered.

REVIEW OF LITERATURE

Prior to the work of Pucher, Vickery, and Wakeman (16), (17), (18), on the quantitative determination of organic acids in plant material, lack of accurate methods led to a dearth of significant data in this field. Since that time papers have begun to appear and their number will undoubtedly continue to grow.

Riddle (20) made an extensive survey of the literature and found that it contains practically no analytical data on the organic acids and inorganic cations occurring in the same plants. From purely theoretical considerations, he predicts that when such data becomes available it will be shown that the quantity occurrence of the inorganic cations within the various plants depends to a large extent on the kind and quantity of the various organic acids within each plant. He assumes that the organic acids orient themselves at membrane interfaces in root hairs in a partially dissociated form. There they are ready to receive incoming cations. If one organic acid dissociates more than another there will be a correspondingly greater number of its anions at the interface, causing a greater cation absorption. Since oxalic acid has a greater dissociation constant than any of the other commonly occurring plant acids, plants containing oxalic acid should have a larger amount of cations. In conjunction with this theory he also assumes a 'salt phase' or layer of salt along the interface. If this interface 'salt phase' is temporarily saturated with a salt as calcium oxalate then no more calcium will combine with the oxalic acid, and if no other acid anion is available then the absorption of calcium is inhibited. This does not prevent the absorption of some other cation whose oxalate is more soluble than calcium oxalate. However if some

other acid, such as propionic, is present at the interface, calcium can combine with it, and since the solubility of calcium propionate is much greater than calcium oxalate, calcium absorption will be enhanced. On the other hand propionic acid is much less dissociated than oxalic acid, and so less of its anions will be available. The result therefore, will be a decrease in calcium absorption. In conclusion, he lists three factors which contribute, in a high degree, to the selective assimilation of cations by plants: (1) the quantity and kind of organic acids specific to the plant, (2) dissociation constants of such organic acids, and (3) solubility of the organic salts formed at the interface by combination with the available incoming cations.

Iljin (10) has analyzed several species of plants from all types of habitats for calcium and organic acids. He found that species vary considerably in their ability to regulate calcium intake. Those species naturally adapted to calcarious soils have a lower calcium content and show some sort of regulatory ability. Other plants not adapted to limestone soils but found growing there may absorb as much as five times the amount of calcium they would ordinarily absorb if grown on their native soil. Plants having a regulatory action toward calcium uptake are lower in total calcium and in total organic acids than those plants with no re ulatory ability. However he found several species of plants which contained no oxalic acid and very small amounts of other organic acids but still had a vigorous precipitating capacity for calcium. He found that citric acid increased markedly with increase in calcium uptake and was correlated to a considerable extent with the soluble calcium in the plant. Malic acid increased in the same manner but to a lesser degree than citric acid. In plants containing large quantities of oxalic acid nearly the entire

amount of calcium in the plant is precipitated, while other plants with little or no oxalic acid have a large proportion of their calcium in a sap soluble state.

Piatnitsky (13) studied the effect of mineral salts on the storage of citric acid by excised leaves of tobacco. Half leaves of Young overgrown seedlings were used. One half of the leaf was dipped in the salt solution and the other half was used as the control. A wide range of K, Mg, and Na, salts were tested. None of the salts used seemed to have a definite predominance over the others as regards its influence on the storage of citric acid by the leaves. However in the case of leaves from adult plants $MgCl_2$ did increase citric acid very considerably. When the tobacco leaves were kept in 0.25 M $MgCl_2$ for 24 hours they decreased considerably in protein, carbohydrates, and especially malic acid but increased greatly in citric acid. The increase in citric acid was practically equivalent to the decrease in malic acid.

Pucher, Vickery, and Wakeman (19) found in tobacco a large excess of positive ions over inorganic anions and this excess of positive ions was positively correlated with the ether soluble organic acids. From this they concluded that in the case of tobacco organic acids occupy a dominating position with respect to the balance of positive and negative ions, and that these acids are closely concerned in the phenomena of inorganic nutrition.

Working with barley roots Hoagland and Broyer (8) found evidence from buffer curves that organic acids were in some way related to the absorption of cations. Ulrich (24) extended this work and determined the total organic acidity of barley roots exposed to different salt solutions. He found that whenever cations were ab-

sorbed in excess of anions the root cells tended to compensate for the potential increase in alkalinity through the formation of organic acids. When anions were absorbed in excess of cations a tendency toward a decrease in organic acids was indicated. The formation of organic acids took place readily as long as sufficient sugars were available. When these sugars were depleted, the ammonia or unstable amides increased until finally the source of energy reflected in these end-products was exhausted to a level at which it could no longer be used for metabolism. Thereafter the organic acids decomposed to supply energy. When more cations than anions were absorbed, a decrease in the respiratory quotient occurred, indicating the formation of organic acids. When more anions than cations were absorbed, the respiratory quotient became greater than one, suggesting the utilization of organic acids in respiration. These observations indicated a close relationship between the acid-base balance in the root cells and their organic acids.

Chandler (3) working with the leaves of several forest trees found in general that the total oxalates were correlated with the total calcium content. More significantly, he found that in all but two species examined the total oxalates were just equivalent to the acetic acid insoluble calcium. From this he concluded that all of the oxalates were present as insoluble calcium oxalate. In none of the species studied did the total oxates exceed the total calcium content. Dunne (6) working with buckwheat inferred that the oxalic acid could be precipitated by potassium as well as calcium, the insoluble potassium salt being in the form of potassium acid oxalate. In contrast to the material used by Chandler (3), buckweat contains oxalic acid in excess of the total calcium.

From a compilation of data on the analyses of a wide variety of plants, Parker and Truog (12) found a close correlation between calcium

and nitrogen content. Potassium, magnesium, and phosphorus did not bear this close relationship to the nitrogen content. The authors considered that the larger the amount of nitrogen the more protein was formed and hence more organic acids, as oxalic, acetic, succinic and formic, were formed. Larger amounts of calcium were then necessary to neutralize these acids. This of course is based on the assumption that proteins are the major source of organic acids in plants. Iljin (10) observed that in several species of plants calcium content seemed to vary directly with the amount of nonalbuminous nitrogen. Chandler (5) found no relationship between the nitrogen and oxalate content of the foliage of trees. It appeared that the production of oxalic acid in the forest trees considered was dependent upon more factors than simply nitrogen content.

Wadleigh and Shive (26) found in corn that plants grown with a mixture of ammonia and nitrate nitrogen contained less organic acids and had a lower base content than plants grown with nitrate as the sole source of nitrogen. Clark (4) working with tomato found essentially the same thing to be true. Vickery and Pucher (25) claim that it is probably a general phenomenon that nitrogen applied as nitrate causes a higher organic acid and inorganic base content than nitrogen applied as ammonium salts. They also state that not only the total amount of organic acids, but the relative proportions of the different acids present are profoundly affected by the form in which the nitrogen is administered to the plant.

MATERIALS AND METHODS

Culture Methods

Twelve species of plants were chosen for this experiment: spinach, variety Summer Savoy; beets, variety Early Superb; wheat, variety Leepland; Kentucky blue grass; alfalfa; lima beans; peas, variety Alaska; soy beans, variety Biloxi; buckwheat; lettuce, variety Grand Rapids; cantaloupe, variety White Seeded Pink Meat; and tomatoes, variety Break of Day.

The seeds of the various species were planted in sand and when the plants had attained sufficient height they were thinned to a few uniform plants per pot. Six pots were allotted to each species of plant.

The 1940 crop was planted the latter part of September and all plants had been harvested by the end of November.

An effort was made to keep the night temperature of the greenhouse between 65° and 70° F.

The following nutrient solution, table I, was applied to all plants at the rate of approximately 1 liter per crock, per 24 hours.

TABLE I

Composition Of Nutrient Solution

Major Elements		Minor Elements	
Salt	Mols per Liter of Nutrient Solution	Salt	Grams per liter of stock Solution
$Ca(NO_3)_2.4H_2O$	.005	$MnCl_2.4H_2O$	.387
KH_2PO_4	.0025	$ZnSO_4.7H_2O$	.097
$MgSO_4.7H_2O$	.0025	H_3BO_3	.309
		$CuSO_4.5H_2O$	.055
			1 cc of stock solution per liter of nutrient solution.

Iron: 10 cc of a 0.5% ferric tartrate solution per 18 liters of nutrient solution.

In 1941 the experiment was repeated, This crop was planted the latter part of January and all plants had been harvested by the middle of April, with the exception of cantaloupe. The cantaloupe was not planted until April so as to benefit by the longer days and greater light intensity.

The planting and treatment of the 1941 crop was the same as that for the 1940 crop.

These experiments were concerned only with the vegetative phase of growth. All plants were harvested before they blossomed or in cases where blossoms appeared before sufficient vegetative growth had been attained the blossoms were pinched off.

A Modified Capillary Drip Method For Use In Solution Culture Work

Numerous methods have been suggested in the literature to accurately control the amounts of solution supplied to culture vessels. In devising such a method certain factors must be kept in mind. The apparatus must be accurate within reasonable limits, simple, easily constructed, and should not require undue labor to keep it functioning. The capillary drip method as originally described by Shive (23) is accurate, but requires a large expenditure of time to keep it functioning. It requires frequent refilling of the individual reservoirs and adjustment of the capillaries after each filling.

The system used in the present experiment retains the capillaries but is so constructed that after the initial adjustment little time is required to keep it working. The apparatus consists of a series of small reservoirs connected by siphons. The culture solution is maintained at a constant level in these reservoirs by an 18 liter bottle filled with solution and inverted into a small cup. The cup is connected to the first reservoir by a siphon. Solution flows from the bottle to the first reservoir and from there it siphons into the next reservior in the series or through the capillaries directly into the culture crocks. The small reservoirs are white enamel basins. Each basin is covered with a round lid made from Celotex. These lids are painted with black asphalt to prevent water absorption. The tops of the lids may be painted white to reflect light and thus keep the temperature of the culture solution down. The lids are notched on the under side to facilitate the introduction of the capillary and siphon tubes. Each basin supplies several crocks, usually four.

The culture crocks are arranged in parallel rows on a suitable table and the basins are set on a shelf about 2-3 inches higher than the tops of the crocks. The capillary and siphon tubes are inserted in the notches in the lid and allowed to dip into the solution in the basins. The capillary tubes are supported by an adjustable support fastened to the shelf at the base of the reservoirs. The capillary tubes, acting as siphons, are then adjusted by the metal support to deliver the required amount of solution to each culture vessel. Figure 1 shows the complete set up.

After the initial adjustment of the capillaries, which can be done with the aid of a stop watch, the only thing required is to keep the 18 liter reservoir full. The capillaries may occasionally become clogged but they can be cleaned by applying suction. If this does not suffice it is a relatively simple matter to have another set of capillaries which may be used to replace the clogged ones.

The shapes and dimensions of the apparatus are shown in figures 2 and 3.

The adjustable support, figure 2, is made from scraps of tin that may be obtained at any plumber's shop. It is made in two pieces and the size governed by the requirements of the set-up. The larger piece A is cut from a strip of tin and small holes drilled near each end. It is then given a half twist so that it can be fastened to the shelf with a screw. Piece B is also cut from a piece of tin and a hole drilled near the center. One end of B is notched and the two flaps twisted to form a Y to support the capillary tube. Piece A and B are then bolted together with a small stove bolt and lock washer to form C.

Fig. 1. Complete set-up of solution culture apparatus.

The bolt acts as a pivot facilitating the raising and lowering of the raising and lowering of the capillary.

The siphon tubes, figure 3, connecting the reservoirs are made from 8 mm (inside diameter) glass tubing. These tubes are first painted black and then a coat of white over the black. This completely eliminates the growth of algae in these tubes.

The capillary tubes, figure 3, are made from 1 mm. capillary tubing. Smaller bore (0.5 mm.) facilitates more accurate adjustment of flow but clogs more easily.

The modified capillary drip method used in the solution culture work has the following advantages:

1. Simple and easily constructed from readily obtainable materials.
2. Accurate for all ordinary work.
3. Labor saving. Requires only the filling of one reservoir and an initial regulation of capillaries.
4. A constant head of pressure is maintained insuring constant flow of the solution at all times.

Analytical Methods

All samples were taken between 9:00 A. M. and 11:00 A. M. Each set of plants was divided into two equal lots and the fresh weight obtained immediately. Where possible both lots were divided into stems and leaves, petioles being included with the stems. The leaves and stems of one lot were placed in separate quart fruit jars, the lid put on tightly and the jars placed in a refrigerator at -15° C. The leaves and stems of the other lot were placed in separate wire baskets, dried in a hot air

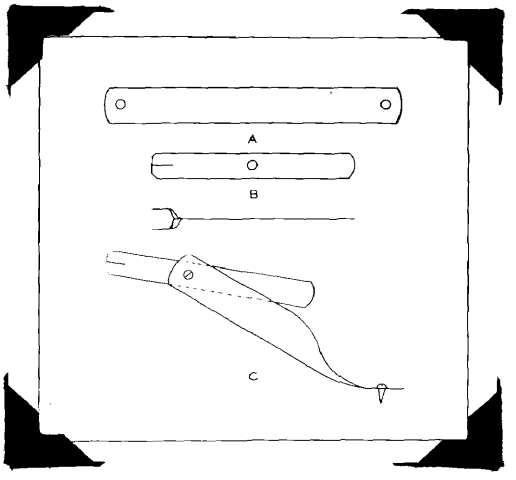


Fig. 2. Diagram of capillary support.

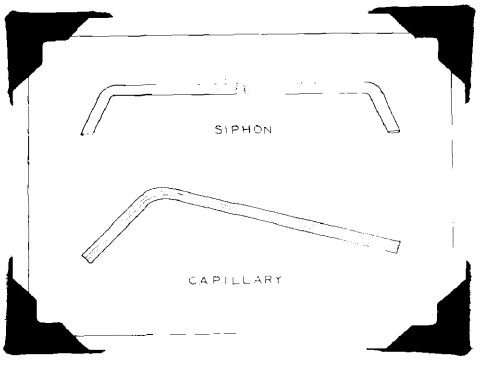


Fig. 3. Diagram of siphon and capillary tube.

oven at 70° C., ground to pass a 60 mesh seive, then stored in aluminum boxes until ready for analysis. The methods of the Association of Official Agricultural Chemists (1) were used for the final determination of calcium, magnesium, potassium, sulphur and nitric acid.

Extraction and Analysis of Juice

The samples put up for juice analysis were frozen at -15° C. for at least 12 hours. After this time they were allowed to stand at room temperature until completely thawed. This usually required 3 to 4 hours. The material was then placed in a stout muslin cloth and subjected to 10,000 pounds pressure in a Carver hydraulic press. The juice was collected in a beaker.

The determination of sap soluble constituents in the extracted juice is based on the work of Sayre and Morris (21), (22).

Specific gravity.—Duplicate 2 ml. samples of the juice were pipetted into tared, glass stoppered, weighing bottles. These were immediately weighed and the specific gravity calculated.

Total solids.—The samples used for specific gravity were dried in a hot air oven at 70° C. then placed in a vacuum oven at 65° C. and 2 - 3 cm. pressure until drying was complete.

Titratable acidity and pH.—Duplicate 5 ml. samples of the juice were pipetted into 50 ml. beakers and titrated to a pH of 8.5, using 1/10 normal sodium hydroxide. The end point was determined with a Beckman pH Meter employing a glass electrode. The pH value of the sap was determined on the samples used for titratable acidity.

Oxalic acid.—Aliquots of 5 to 10 ml. of juice, depending upon the oxalic acid content, were pipetted into 25 ml. volumetric flasks and made to volume. Duplicate 10 ml. aliquots of the diluted juice were measured into

100 ml. beakers and 0.5 N HCl added until slightly acid to congo red. This was allowed to stand overnight and any material that flocculated was filtered off on an asbestos mat. The coagulum was washed 2 or 3 times with small amounts of water slightly acidified with HCl. Two drops of methyl red indicator were added to the filtrate ammonia diluted 1-4 added until faintly alkaline, then 2-3 ml. of glacial acetic acid followed by 5 ml. of 10 per cent calcium chloride. This was allowed to stand at least 4 hours, when the precipitated calcium oxalate was filtered off in a Gooch crucible and washed with a little very dilute ammonia (1-50). The crucible and contents were transferred to the 100 ml. beaker, 5 ml. of 50 per cent sulphuric acid and 30-40 ml. of water added. The solution was heated to 90° C. and titrated with .02 N potassium permanganate.

This method is essentially the one employed by Pucher, Vickery, and Wakeman (16) in determining the oxalic acid in the ether extract of dried tissue.

Calcium. —Duplicate 10 ml. samples of the juice were measured into small porcelain crucibles and dried in a hot air oven at 70° C. The material was moistened with a few drops of sulphuric acid (1-10) and heated gently over a low flame until thoroughly charred. The crucibles were then placed in a muffle furnace and ashed for 4 hours at 600° C. The addition of sulphuric acid was necessary to eliminate explosion of the material during ashing.

The ash was dissolved in dilute HCl, taken to dryness to dehydrate any silica, redissolved in dilute HCl and made to a volume of 25 ml. Calcium was determined on 10 ml. aliquots of this solution by the micro method (1).

Analysis for Total Constituents

Moisture.---Immediately after cutting the plants, duplicate 15-20 gram samples of leaves and stems were snipped into tared aluminum boxes. The samples were dried to constant weight in a vacuum oven at 65° C. and 2-3 cm. pressure.

Ash. Duplicate 3/4 gram samples of the dried, finely ground material were weighed into tared platinum crucibles. The material was moistened with 25 drops of sulphuric acid (1-10), dried in an oven at 95° C., then placed in a muffle furnace and ashed 4 hours at 600° C. The sulphuric acid was necessary to avoid explosion of the material while ashing.

Calcium. The residue from the ash determination was used for the determination of calcium. The method used was identical with that employed for the analysis of calcium in the juice, except that the volume of the ash solution was 50 ml.

Magnesium. The filtrate and washings from the calcium determination did not contain sufficient magnesium to permit an accurate determination of this constituent. A larger aliquot, 20 ml., was used. This was treated in the same way as the calcium determination and magnesium determined on the filtrate and washings.

Potassium. Duplicate 10 ml. aliquots of the ash solution were used for the determination of potassium. The rapid method was used (1).

Phosphorus. A separate ashing was necessary for the determination of phosphorus and sulphur. Duplicate 1.0 gram samples were weighed into large porcelain crucibles and ashed by the magnesium nitrate method (1). The ash was diluted to 250 ml. and 5 ml. aliquots of this solution used

for the determination of phosphorus by the method of Fiske and Subbarow (7). An Aminco photometer was used for the comparisons. A standardization curve was constructed, using varying concentrations of pure KH_2PO_4. It was thus possible to read the unknown in the colorimeter and find its concentration directly from the standardization curve.

Sulphur. Duplicate 200 ml. aliquots of the ash solution were used for the sulphur determination (1).

Total organic acids. The method used was that of Pucher, Vickery, and Wakeman (16).

Duplicate 2.0 gram samples of the dry, finely ground tissue were acidified with 4 N sulphuric acid and extracted in a Soxhlet with ether. The extract was washed with dilute sodium hydroxide and the ether carefully distilled. The residue was then made to a volume of 100 ml. with carbon dioxide free water. Duplicate 10 ml. aliquots were titrated electrometrically to determine the total organic acidity.

Oxalic and citric acids. The method used was that of Pucher, Vickery, and Wakeman (16), (17).

Malic acid. The uranium acetate method of Dunbar and Bacon (5), modified by Vickery and Pucher (15) was used. Admitedly this method is not as good as the one developed later by Pucher, Vickery, and Wakeman (16) but the time saved seemed to justify its use.

Duplicate 20 ml aliquots of the organic acid solution were measured into 25 ml. volumetric flasks, a drop of phenolphthalein was added, followed by 2.5 N sodium hydroxide until the reaction was just alkaline. It was then acidified with 0.5 ml. of glacial acetic acid. Three grams of powdered uranium acetate and a little Norite were added and the flask shaken on a mechanical shaker for 1 hour. The solution

was made to volume with saturated uranium acetate solution, thoroughly mixed and filtered into a 2 decimeter polarimeter tube. The rotation was observed in a Haensch and Schmidt saccharimeter. Twenty separate readings were taken and the average used. The grams of malic acid in 25 ml. of solution secured from the aliquot of original solution taken is equal to :-

$$V^{\circ} \times \frac{0.033}{4}$$

Nitric acid. The method used for the extraction of the organic acids also extracted the nitric acid quantitatively. A suitable aliquot, usually 2 ml., of the organic acid extract was placed in a 300 ml. Kjeldahl flask, the nitrate reduced with sulphuric acid and reduced iron powder, the ammonia distilled and Nesslerized (1). Readings were taken in an Aminco photometer and the quantity of nitrogen read from a standardization curve. Varying concentrations of a standard ammonium sulphate solution were used to construct the standardization curve.

EXPERIMENTAL RESULTS

At the time of harvest the heights of the plants and fresh weights were recorded. Where possible the number of plants per sample was also obtained. These data as well as the age of the plants at time of harvest are recorded in tables II and III.

TABLE II

Growth data (1940 crop)

Plant	Plant date	Harvest date	Age	No. of plants	Total Fresh wt.	Average height	Av. wt. per plant
			days		grams	inches	grams
Spinach	Oct.3.	Dec. 6	63	18	438	6	24.3
Beets	Oct.2	Nov. 30	58	16	430	14	26.9
Wheat	Oct.2	Nov. 2	30	106	406	14	3.8
Blue grass	Sept.28	Nov. 12	54	---	190	6	---
Alfal.	Oct.2	Dec. 11	69	---	368	12	---
Lima beans	Oct.2	Nov. 11	39	12	403	33	34.
Peas	Sept.28	Nov. 2	34	30	172	27	5.7
Soy beans	Sept.28	Oct. 31	32	30	455	15	15.2
Buckwheat	Sept.28	Oct. 31	32	36	381	19	10.6
Lettuce	Oct.3	Nov. 22	49	23	227	7	9.8
Cantaloupe	Oct.2	Nov. 20	48	18	478	—	26.6
Tomato	Oct.3	Nov. 28	55	15	1447	16	96.5

TABLE III

Growth data (1941 crop)

Plant	Plant. date	Harvest date	Age	No. of Plants	Total Fresh wt.	Av. Height	Av. wt. per plant.
			days		grams	inches	grams
Spinach	Jan. 31	Apr. 5	63	30	769	7±	25.6
Beets (tops)	Jan. 31	Mar. 27	54	24	780	14±	32.5
Wheat	Jan. 31	Mar. 5	32	108	302	11±	2.8
Blue grass	Jan. 31	Mar. 21	48	---	182	7±	---
Alfalfa	Jan. 31	Apr. 8	66	---	424	13±	---
Lima beans	Feb. 25	Apr. 11	44	18	679	22±	38
Peas	Jan. 31	Mar. 10	37	62	368	27±	5.9
Soy beans	Feb. 25	Apr. 2	37	30	475	13±	15.8
Buck-wheat	Jan. 31	Mar. 10	37	60	448	15±	7.5
Lettuce	Jan. 31	Mar. 21	48	48	268	6±	5.6
Canta-loupes	Apr. 6	May 14	37	18	934	---	51.9
Tomatoes	Jan. 31	Apr. 6	64	12	1203	16±	100.2

It can be seen from the average fresh weight per plant that the two crops are fairly comparable with the exception of cantaloupe. The 1940 crop of cantaloupe was grown in the late fall when light intensity was low and consequently there was less growth than in the 1941 crop which was grown in April and May when light intensity was increasing. Regardless of this great difference in the amount of growth of the two crops, analyses based on dry weight showed them to be quite comparable.

In the case of alfalfa and blue grass the great number of plants made it inadvisable to attempt a count as the plants would lose large

quantities of moisture during the process. This would lead to serious error in latter determination.

Inorganic Cations and Anions

Table IV gives the inorganic cations in milliequivalents per 100 grams of dry tissue in the different plants for the 1940 crop and table V the inorganic cations and anions for the 1941 crop. The inorganic anions were not determined in the 1940 crop. It can be seen that the inorganic ion content is quite comparable in the two crops. The data on the cations of the 1941 crop are shown more clearly in figure 4. Figure 5 shows graphically the distribution of calcium. Since the 1940 crop was similar it is not included.

TABLE IV.

Inorganic cations
(1940 crop)

Plant		Milliequivalents per 100 grams of dry tissue				
		Total magnesium	Total potassium	Total c calcium	Soluble calcium	Soluble calcium as percent of total
Lima beans	leaves	74.0	111.2	117.2	80.5	68.7
	stems	64.6	167.3	81.7	57.5	70.5
Peas	leaves	52.6	78.9	128.0	—	—
	stems	34.5	115.1	59.0	—	—
Alfalfa	leaves	56.7	80.6	132.6	69.6	52.5
	stems	42.8	92.7	45.6	16.3	35.8
Soy beans	leaves	93.5	88.5	112.3	55.8	49.7
	stems	85.5	146.1	80.3	38.8	48.3
Beets	leaves	240.6	176.6	99.0	2.3	2.4
	petioles	92.9	348.5	27.8	4.7	17.2
Spinach	leaves	165.3	186.5	127.6	1.3	1.0
	petioles	103.0	333.7	74.3	2.0	2.8
Buckwheat	leaves	188.5	61.9	146.6	4.8	3.3
	stems	110.8	243.0	105.6	13.6	12.9
Blue grass	leaves	52.6	155.1	24.6	20.3	82.6
Wheat	leaves	52.1	191.5	22.6	17.0	75.3
Tomatoes	leaves	114.3	102.0	183.0	91.0	49.8
	stems	129.4	228.0	104.3	33.8	32.4
Lettuce	leaves	59.2	216.9	81.6	48.7	59.6
Cantaloupe	leaves	125.0	96.3	291.3	45.7	15.7
	stems	93.2	216.9	79.6	46.0	57.8

Inorganic anions and cations

1941 Crop

Plant		Milliequivalents per 100 grams of dry tissue: total NO_3^-	total SO_4	Total H_2PO_4	Total magnesium	Total Potassium	Total Calcium	Soluble calcium	Soluble calcium as % of total
Lima beans	leaves	20.4	18.5	13.1	69.6	98.9	118.3	90.8	76.8
	stems	32.3	9.5	15.2	48.5	125.7	75.0	31.5	42.1
Peas	leaves	19.5	25.9	23.9	47.8	77.8	105.5	86.5	82.1
	stems	48.7	12.8	15.3	33.7	114.4	66.0	43.1	65.3
Alfalfa	leaves	24.6	34.4	19.4	41.4	93.4	109.0	53.9	49.4
	stems	29.6	15.1	21.8	33.4	123.6	49.7	18.4	37.3
Soy beans	leaves	15.0	23.0	24.9	71.0	76.6	128.7	71.6	55.7
	stems	32.1	23.3	23.6	58.1	104.2	81.0	36.0	44.4
Beets	Leaves	22.3	30.0	24.9	201.0	151.7	112.0	1.3	1.2
	Petioles	157.5	12.4	15.2	65.3	261.0	42.3	2.7	8.9
Spinach	leaves	40.4	30.5	39.7	137.3	186.7	133.7	1.1	0.9
	Petioles	100.3	22.2	37.9	85.2	242.1	51.3	2.6	5.2
Buck-Wheat	leaves	17.7	21.0	27.1	174.9	57.8	157.0	2.8	1.8
	stems	162.5	12.2	22.1	92.1	186.7	106.0	13.5	12. 7
Blue grass	leaves	74.3	26.7	23.4	47.7.	142.2	23.0	17.0	75.7
Wheat	leaves	76.9	30.0	35.7	41.7	178.1	22.7	15.0	66.7
Tomatoes	leaves	20.4	91.8	30.0	105.3	82.4	191.7	116.8	60.9
	stems	106.4	15.2	29.7	119.3	181.4	112.0	39.7	35.3
Lettuce	leaves	54.3	24.8	26.3	50.5	195.2	60.7	47.5	78.5
Cantaloupe	leaves	44.3	72.4	26.9	148.6	72.6	336.7	51.0	15.2
	stems	120.7	27.4	26.1	81.1	148.0	91.7	59.8	65.5

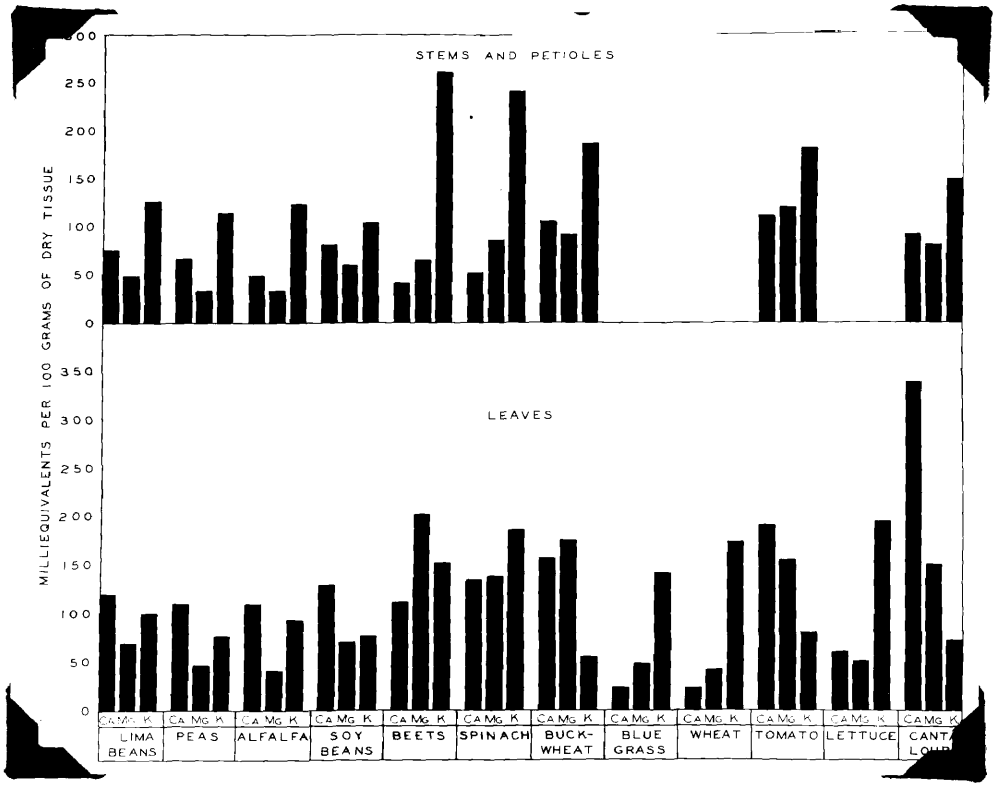


Fig. 4. Distribution of Ca, Mg, and K in leaves and stems of the plants tested (1941 crop).

It is clearly shown that when different species of plants are all grown under the same conditions of nutrient supply they take up inorganic ions in varying proportions according to inherent characteristics of the plant. It should be noted that even plants in the same families as the legumes - soy beans, lima beans, alfalfa, and peas; the Chenopodiacae- beets and spinach; the grasses - blue grass and wheat, all tend to accumulate cations in the same relative proportions. These results corroborate those of Newton (11).

Calcium and magnesium tend to accumulate in greater quantities in the leaf blade tissue than in the stems and petioles. The reverse is true of potassium. Much larger quantities of this element accumulates in the stems and petioles than in the leaf blades. The exceedingly high amount of calcium present in cantaloupe leaves is of special interest and will be considered later.

The soluble calcium as per cent of total calcium ranges from 70 - 80 percent in some plants down to 1 percent in others.

Tables VI and VII give the percentage composition of the various inorganic constituents for the two crops 1940 and 1941. The percentages of ash appear very high. This is due to the necessity of treating the dry tissue with sulphuric acid before ashing. The quantities of ash are therefore reported as sulfated ash. All samples were treated the same so comparison of relative amounts are valid with this experiment.

Organic acids

Tables VIII and IX give the various organic acids in milliequivalents per 100 grams of dry tissue in the two crops 1940 and 1941. Tables X and XI give the same data calculated as percentage of wet and dry weight. A word of warning is necessary when comparing the data in this experiment

TABLE VI

Percentage Composition of inorganic constituents
(1940 crop)

Plant		Ash (sulfated)		Calcium		Magnesium		Potassium	
		Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
Lima beans	Leaves	2.70	20.71	0.306	2.34	0.117	0.900	0.567	4.35
	stems	2.31	22.03	0.171	1.63	0.082	0.786	0.686	6.54
Peas	Leaves	2.95	18.12	0.417	2.56	0.104	0.640	0.503	3.09
	stems	2.09	15.48	0.159	1.18	0.057	0.420	0.608	4.50
Alfalfa	Leaves	3.00	19.07	0.417	2.65	0.109	0.690	0.496	3.15
	stems	2.42	13.09	0.169	0.913	0.096	0.520	0.671	3.63
Soy beans	Leaves	2.66	18.29	0.327	2.25	0.166	1.14	0.504	3.46
	stems	2.14	20.43	0.168	1.61	0.109	1.04	0.599	5.71
Beets	Leaves	3.06	32.75	0.185	1.98	0.273	2.93	0.645	6.91
	petioles	2.28	35.76	0.035	0.547	0.072	1.13	0.869	13.63
Spinach	leaves	3.80	33.16	0.293	2.55	0.231	2.01	0.837	7.29
	petioles	3.29	39.31	0.125	1.49	0.105	1.25	1.09	13.05
Buck-wheat	Leaves	3.36	23.97	0.411	2.93	0.321	2.29	0.339	2.42
	stems	1.89	32.95	0.108	2.11	0.069	1.35	0.486	9.50
Blue grass	Leaves	2.93	17.28	0.084	0.493	0.108	0.640	1.028	6.07
Wheat	Leaves	2.03	19.58	0.047	0.453	0.066	0.633	0.776	7.49
Tomatoes	Leaves	2.69	25.91	0.381	3.66	0.145	1.39	0.415	3.99
	stems	1.76	32.52	0.113	2.09	0.085	1.57	0.481	8.91
Lettuce	Leaves	1.42	28.56	0.081	1.633	0.036	0.720	0.422	8.48
Cantaloupe	Leaves	3.77	34.43	0.637	5.83	0.166	1.52	0.412	3.77
	stems	1.58	28.83	0.087	1.59	0.062	1.13	0.465	8.48

TABLE VII

Percentage composition of inorganic constituents
(1941 crop)

Plant		Ash (sulfated)		Calcium		Magnesium		Potassium		Nitrate nitrogen		Sulphur		Phosphorus	
		Dry wt. %	Wet wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %
Lima beans	leaves	20.28	2.70	0.315	2.36	0.113	0.896	0.515	3.87	0.053	0.397	0.039	0.296	0.054	0.405
	stems	16.72	2.17	0.195	1.50	0.076	0.590	0.639	4.93	0.059	0.452	0.020	0.153	0.061	0.470
Peas	leaves	18.35	2.40	0.330	2.11	0.091	0.581	0.477	3.04	0.043	0.273	0.065	0.414	0.116	0.740
	stems	14.33	1.84	0.170	1.32	0.052	0.410	0.575	4.47	0.088	0.682	0.026	0.205	0.061	0.475
Alfalfa	leaves	16.35	2.68	0.358	2.18	0.082	0.503	0.599	3.65	0.056	0.345	0.090	0.550	0.098	0.600
	stems	16.04	2.47	0.153	0.993	0.062	0.406	0.745	4.83	0.064	0.412	0.037	0.242	0.104	0.675
Soy beans	leaves	14.64	3.05	0.422	2.57	0.141	0.863	0.489	2.986	0.034	0.210	0.060	0.369	0.126	0.770
	stems	16.95	2.05	0.196	1.62	0.085	0.706	0.492	4.073	0.054	0.450	0.045	0.374	0.088	0.730
Beets	leaves	31.44	5.40	0.242	2.24	0.265	2.45	0.642	5.93	0.34	0.313	0.052	0.481	0.083	0.770
	petioles	32.19	2.17	0.053	0.846	0.049	0.793	0.639	10.21	0.138	2.21	0.012	0.198	0.029	0.470
Spinach	leaves	32.68	3.34	0.273	2.67	0.170	1.67	0.746	7.30	0.058	0.565	0.050	0.488	0.126	1.23
	petioles	29.85	2.29	0.079	1.03	0.079	1.04	0.727	9.47	0.108	1.40	0.027	0.355	0.090	1.18
Buck-wheat	leaves	25.39	3.28	0.406	5.14	0.275	2.13	0.292	2.26	0.032	0.247	0.043	0.336	0.109	0.840
	stems	29.63	1.77	0.127	2.12	0.067	1.12	0.436	7.30	0.136	2.28	0.011	0.195	0.041	0.685
Blue grass	leaves	17.16	2.62	0.070	0.460	0.088	0.580	0.847	5.56	0.158	1.04	0.065	0.427	0.110	0.725
Wheat	leaves	18.99	2.19	0.052	0.433	0.058	0.506	0.780	6.77	0.124	1.08	0.055	0.480	0.127	1.11
Tomatoes	leaves	23.64	2.63	0.427	3.83	0.142	1.28	0.358	3.22	0.032	0.285	0.164	1.47	0.104	0.930
	stems	29.52	1.75	0.133	2.24	0.086	1.45	0.420	7.09	0.088	1.49	0.014	0.243	0.054	0.920
Lettuce	leaves	25.57	1.67	0.079	1.21	0.040	0.613	0.498	7.63	0.049	0.760	0.259	0.398	0.126	0.770
Canta-loupe	leaves	36.65	4.34	0.798	5.73	0.214	1.81	0.337	2.84	0.073	0.620	0.137	1.16	0.099	0.835
	stems	25.53	1.49	0.116	1.83	0.062	0.966	0.367	5.79	0.011	1.69	0.028	0.439	0.051	0.810

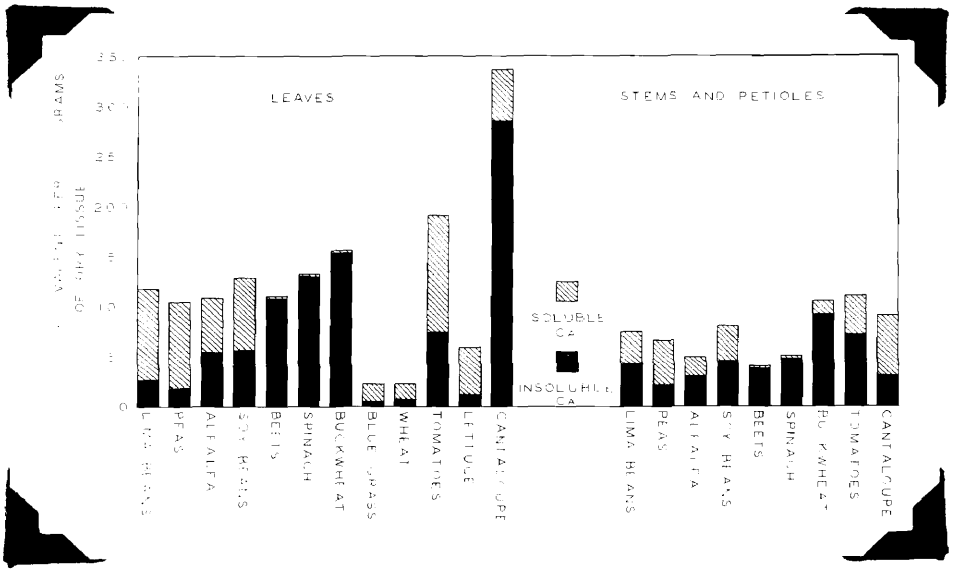


Fig. 5. Relative amounts of soluble and insoluble calcium in leaves and stems of the plants tested (1941 crop)

With data from plants grown under different conditions. As Clark (4) and Wadleigh and Shive (26) have shown and as Vickery and Pucher (25) have stated the kinds and amounts of organic acids occurring in plants are profoundly influenced by the form in which nitrogen is supplied to the plant.

It is evident, that in many cases it is possible to account for only a small amount of the total organic acidity as oxalic, malic and citric acids. In wheat and blue grass the unknown organic acids make up 70 - 80 percent of the total. In beets, spinach, and buckwheat 70 - 85 percent of the total acids can be accounted for. Data of this nature clearly show that there is still much to be done in the way of developing methods for the determination of the acids of the unknown group.

Cation-Anion Balance

Pucher, Vickery, and Wakeman (19) have shown in tobacco grown under controlled fertilizer conditions, a larger excess of positive ions over inorganic anions and this excess is positively correlated with the ether soluble organic acids. An attempt has been made in the present investigation to determine if this is a common phenomenon among plants in general.

In the analyses no attempt was made to fractionate organic and inorganic sulphur and phosphorus. In the calculations of milli-equivalents of inorganic anions all sulphur was considered as sulfate and all phosphorus as phosphate. This is not strictly true as phosphorus and sulphur are known to enter into many organic compounds as proteins, phosphatides, hexose phosphates, etc. However it seems safe to assume that the major portions of these elements are in the inorganic form in the plant. All phosphorus was calculated as the mono-

TABLE VIII

Organic acids. (1940 crop)

Plant		Milliequivalents per 100 grams of dry tissue					Unknown as percent of total.
		Total acids	Oxalic	Malic	Citric	Unknown acids	
Lima beans	leaves	237.2	30.2	24.9	38.5	143.6	60.5
	stems	259.3	29.6	30.2	32.2	167.3	64.5
Peas	leaves	255.7	17.0	56.1	86.0	96.6	37.8
	stems	219.6	6.0	47.0	56.0	110.6	50.4
Alfalfa	leaves	199.5	30.0	24.9	22.9	121.7	61.0
	stems	166.8	9.8	16.8	10.9	129.3	77.5
Soy beans	leaves	246.1	17.4	1.3	58.0	169.4	68.8
	stems	235.5	21.8	26.2	20.6	166.9	70.9
Beets	leaves	405.9	298.0	21.2	20.6	66.1	16.3
	petioles	201.8	86.6	40.0	22.4	52.8	26.2
Spinach	leaves	362.1	280.4	6.5	3.7	71.5	19.7
	petioles	238.5	150.9	16.8	4.9	65.9	27.6
Buckwheat	leaves	353.6	253.8	15.8	26.4	57.6	16.3
	stems	207.2	109.2	16.8	13.4	67.8	32.7
Blue grass	leaves	142.7	0.0	7.7	26.3	108.7	76.2
Wheat	leaves	133.5	0.0	16.4	6.5	110.6	82.8
Tomatoes	leaves	239.7	69.3	30.9	53.8	85.7	35.7
	stems	220.5	82.1	52.7	10.7	75.0	34.0
Lettuce	leaves	197.2	2.3	72.1	18.6	104.2	52.8
Cantaloupe	leaves	85.6	0.0	22.2	34.0	29.4	34.3
	stems	129.7	0.0	53.7	10.7	65.3	50.3

TABLE IX

Organic acids. (1941 crop)

Plant		Milliequivalents per 100 grams of dry tissue					Unknown as percent of total.
		Total acids	Oxalic	Malic	Citric	Unknown acids	
Lima beans	leaves	249.7	16.9	65.8	48.6	118.4	47.4
	stems	171.1	51.1	24.5	53.1	82.4	48.2
Peas	leaves	212.9	15.1	42.6	80.8	74.4	34.9
	stems	181.0	7.3	46.5	34.5	92.7	51.2
Alfalfa	leaves	185.7	14.1	21.8	14.5	135.3	72.9
	stems	140.7	4.9	30.9	8.4	96.5	68.6
Soy beans	leaves	248.7	15.9	10.6	104.6	117.6	47.3
	stems	177.0	18.9	10.9	26.8	120.4	68.0
Beets	leaves	427.5	322.9	13.4	32.0	59.2	13.8
	petioles	203.1	97.8	28.9	24.8	51.6	25.4
Spinach	leaves	380.4	309.0	8.9	10.5	52.0	13.7
	petioles	226.2	110.2	60.1	9.6	46.3	20.5
Buckwheat	leaves	360.1	265.7	14.1	13.8	66.5	18.5
	stems	205.6	110.4	34.1	6.6	54.5	26.5
Blue grass	leaves	108.5	0.0	9.1	27.8	71.6	66.0
Wheat	leaves	122.1	0.0	18.3	9.6	94.2	77.2
Tomatoes	leaves	269.8	45.2	61.1	74.4	89.1	33.0
	stems	231.6	81.3	89.3	12.3	48.7	21.0
Lettuce	leaves	220.8	1.4	109.0	32.2	78.2	35.4
Cantaloupe	leaves	99.2	0.0	26.2	19.4	53.6	54.0
	stems	119.4	0.0	54.4	5.9	59.1	49.5

TABLE X

Percentage composition of organic acids.
(1940 crop)

Plant		Oxalic acid		Malic acid		Citric acid	
		Wet wt. %	Dry wt. %	wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %
Lima beans	leaves	0.18	1.36	0.22	1.67	0.32	2.47
	stems	0.14	1.33	0.21	2.03	0.22	2.06
Peas	leaves	0.13	0.77	0.61	3.76	0.90	5.51
	stems	0.04	0.27	0.43	3.15	0.49	3.59
Alfalfa	leaves	0.21	1.35	0.26	1.67	0.23	1.47
	stems	0.08	0.44	0.21	1.13	0.13	0.70
Soy beans	leaves	0.11	0.78	0.01	0.09	0.56	3.81
	stems	0.10	0.98	0.18	1.76	0.14	1.32
Beets	leaves	1.25	13.41	0.13	1.42	0.12	1.32
	petioles	0.25	3.90	0.17	2.68	0.09	1.44
Spinach	leaves	1.45	12.62	0.05	0.44	0.03	0.24
	petioles	0.57	6.79	0.09	1.13	0.03	0.31
Buckwheat	leaves	1.60	11.42	0.15	1.06	0.24	1.69
	stems	0.25	4.91	0.06	1.13	0.04	0.86
Blue grass	leaves	0.0	0.0	0.09	0.52	0.29	1.68
Wheat	leaves	0.0	0.0	0.11	1.10	0.04	0.43
Tomatoes	leaves	0.32	3.12	0.22	2.07	0.36	3.44
	stems	0.20	3.70	0.19	3.53	0.04	0.69
Lettuce	leaves	0.005	0.10	0.24	4.83	0.06	1.19
Cantaloupe	leaves	0.0	0.0	0.16	1.49	0.24	2.18
	stems	0.0	0.0	0.20	3.60	0.04	0.69

TABLE XI

Percentage composition of organic acids
(1941 crop)

Plant		Oxalic acid		Malic acid		Citric acid	
		Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %	Wet wt. %	Dry wt. %
Lima beans	leaves	0.10	0.76	0.59	4.41	0.42	3.11
	stems	0.18	1.40	0.21	1.64	0.27	2.12
Peas	leaves	0.11	0.68	0.45	2.86	0.81	5.17
	stems	0.04	0.33	0.40	3.12	0.28	2.21
Alfalfa	leaves	0.10	0.64	0.24	1.46	0.15	0.93
	stems	0.03	0.22	0.32	2.07	0.08	0.54
Soy beans	leaves	0.12	0.71	0.12	0.71	1.10	6.70
	stems	0.11	0.87	0.09	0.73	0.21	1.71
Beets	leaves	1.57	14.58	0.10	0.90	0.22	2.05
	petioles	0.28	4.40	0.12	1.94	0.10	1.59
Spinach	leaves	1.42	13.91	0.06	0.06	0.07	0.67
	petioles	0.38	4.96	0.31	4.03	0.05	0.62
Buckwheat	leaves	1.55	11.96	0.12	0.95	0.11	0.89
	stems	0.30	4.97	0.14	2.29	0.03	0.43
Blue grass	leaves	0.0	0.0	0.09	0.61	0.27	1.78
Wheat	leaves	0.0	0.0	0.14	1.23	0.07	0.62
Tomatoes	leaves	0.23	2.03	0.46	4.10	0.54	4.76
	stems	0.22	3.66	0.35	5.99	0.05	0.78
Lettuce	leaves	0.004	0.06	0.48	7.30	0.13	2.06
Cantaloupe	leaves	0.0	0.0	0.21	1.76	0.15	1.24
	stems	0.0	0.0	0.23	3.65	0.02	0.38

phosphate ion as this is practically the only form in which it can exist at the pH characteristic of the sap of the plants tested.

Table XII present the relationship between the excess inorganic cations and ether soluble organic acids in the leaves and stems of the 1941 crop. Figure 6 presents the data of table XII in graphic form. In the case of the leaf tissue the two components of the graph are nearly superimposable.

TABLE XII

Correlation between excess inorganic cations and total organic acids. (1941 crop)

Plant	Milliequivalents per 100 grams of dry tissue							
	Leaves				Stems and Petioles			
	Total Ca, Mg, K	Total inorganic anions *	Excess Cations **	Total organic acids	Total Ca, Mg, K	Total inorganic anions	Excess Cations	Total Organic acids
Lima beans	286.8	59.9	226.9	249.7	249.2	57.0	192.2	171.1
Peas	231.2	69.2	162.0	212.9	214.1	76.9	137.2	181.0
Alfalfa	243.8	78.4	165.4	185.7	205.7	66.5	139.2	140.7
Soy beans	276.2	62.9	213.3	248.7	243.3	79.0	164.3	177.0
Beets	465.5	77.2	388.3	427.5	368.5	185.0	183.5	203.1
Spinach	457.7	110.5	347.2	380.4	378.7	160.4	218.3	226.2
Buck-wheat	389.7	65.8	323.9	360.1	384.8	196.8	188.0	205.6
Blue grass	212.9	124.3	88.6	108.5				
Wheat	237.0	142.6	94.7	122.1				
Tomatoes	379.2	142.1	237.1	269.8	412.7	151.3	261.4	231.6
Lettuce	306.3	105.4	200.9	220.8				

* NO_3^-, SO_4^{--}, $H_2PO_4^-$ ** Total cations minus inorganic anions

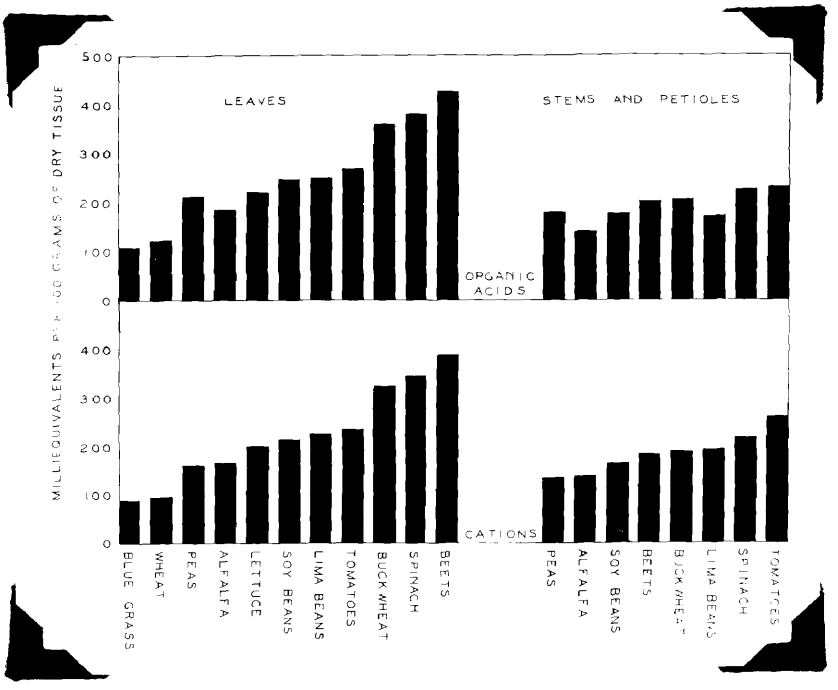


Fig. 6. Correlation between excess cations and total organic acids in leaves and stems of the plants tested. (1941 crop).

As far as the author is aware these are the first data to show that inorganic cations and ether soluble organic acids are positively correlated in a series of different species of plants. The correlation in the leaf blade tissue is exceedingly high (+.990), that in the stem and petiole tissue not as high (+.798). These data indicate strongly that excess positive ions in many plants and particularly in leaf tissue are closely concerned with balancing the organic acids.

It will be noted that cantaloupe is not included. This plant has the highest cation content but the lowest organic acid content, of any of the plants tested. It is obvious that organic acids do not play an important role in balancing cations in this plant. It is entirely possible however, that cations and organic acids are positively correlated, but at a much lower level in this plant than in the other plants tested. The exceedingly high amount of calcium present in the leaves of cantaloupe is rather unusual. Approximately 85 percent of it is insoluble but it is not obvious in what combination it exists. There is no oxalic acid in cantaloupe so the possibility of insoluble calcium oxalate in the plant is excluded. The pH of the expressed sap is unusually high, 7.4 - 7.6. This immediately suggests the possibility that part of the cations are tied up as bicarbonate. Preliminary experiments however do not indicate a very large amount of CO_2 in the sap. Under more careful conditions this may not prove to be the case. Work is now in progress to determine what substances other than phosphate, sulfate, nitrate, and organic acids are binding the large quantities of cations in this plant.

Iljin (10) and Riddle (20) have considered the possibility that the selective absorption of cations by plants is tied up with occurrence of various organic acids that exert an influence on the absorption of a

particular cation over other cations.

Iljin (10) found in general, that soluble calcium in plants was positively correlated with malic and citric acid content.

Figures 7 and 8 compare graphically the amounts of determined organic acids as well as the unknown group with the amounts of cations found in the various plants of the 1941 crop. No striking relationships are evident. Malic and citric acids are found to be somewhat correlated with soluble calcium, particularly in the leaf tissue. Citric acid and those acids of the unknown group show a small negative correlation with total magnesium content in the stems and petioles.

Riddle postulated that when data are available it will be found that cation absorption is correlated with the occurrence of specific organic acids in the plants. His hypothesis is based on the dissociation constants of the acids and the solubilities of the salts formed. Since in many plants it was possible to determine only 25 - 30 percent of the organic acids, it is not possible to put Riddle's hypothesis to a test. However in spinach, beets, and buckwheat where the major portion of the organic acids can be accounted for, some support for Riddle's hypothesis is evident.

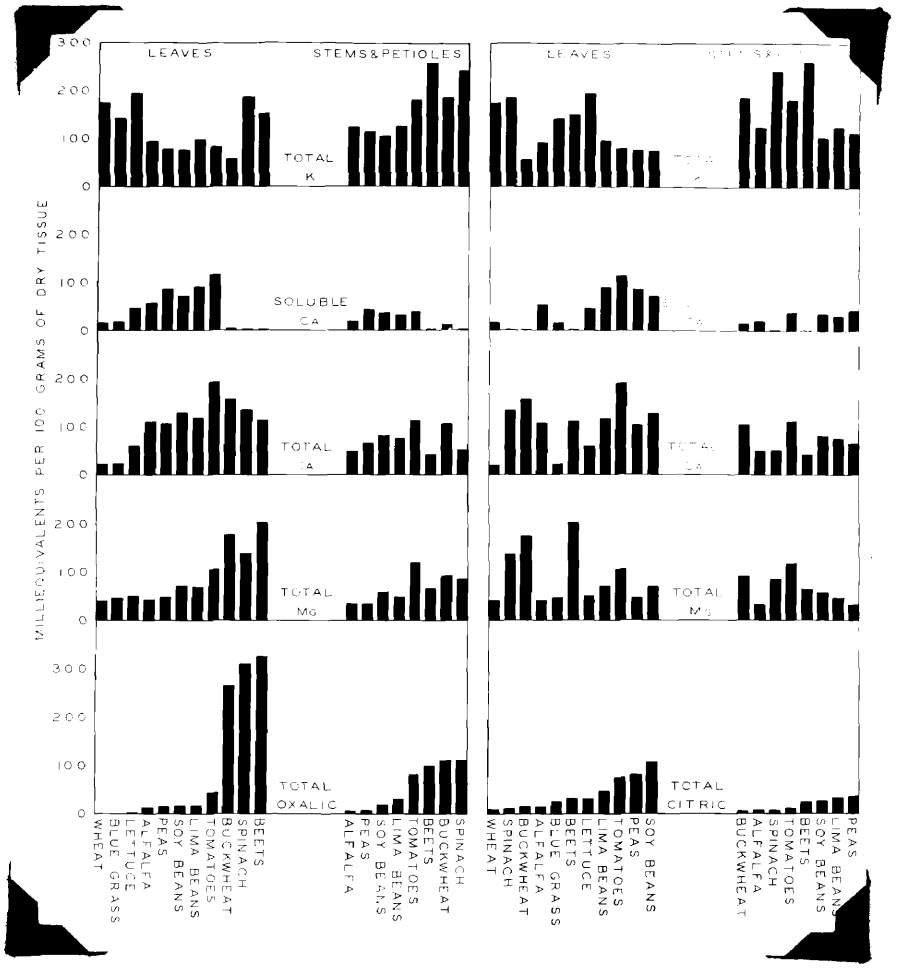
As presented earlier Riddle postulated that plants rich in acid such as oxalic would show a depressed calcium uptake with the consequent effect that some other cation, the oxalate of which is more soluble than calcium oxalate, would be taken up in large quantities. In buckwheat, spinach, and beets a large portion of the total organic acidity is made up of oxalic acid. Figure 7 shows that in the leaves of these plants total calcium decreases as total oxalic acid increases. Total magnesium content is considerably higher in these plants than in the others, indicating enhanced magnesium absorption. Magnesium oxalate is more sol-

uble than calcium oxalate and too it tends to form rather stable supersaturated solutions. According to Middle's hypothesis the decreased calcium uptake is due to the cell sap becoming quickly saturated with the difficultly soluble calcium oxalate and hence calcium absorption is temporarily stopped. Since magnesium is available and it forms a more soluble salt with oxalic acid, the absorption of magnesium is enhanced.

Middle's hypothesis is certainly interesting and when it is possible to obtain a more complete picture of the kinds and amounts of organic acids in various plants, further evidence substantiating his views many be found.

Results of the present experiment indicate that those plants which produce oxalic acid have very small quantities of insoluble calcium, figures 7 and 9. The major portion of the calcium is in a sap soluble state. There are exceptions to this such as cantaloupe. In the plants that produce some oxalic acid but no large quantity all of the oxalic acid is precipitated, presumably as calcium oxalate. However the major portion of the calcium is still in a sap soluble state. Those plants as beets, spinach, and buckwheat which produce large quantities of oxalic acid have practically all of the calcium in an insoluble state, only traces of sap soluble calcium occurring. In addition these plants have considerable quantities of oxalates dissolved in the cell sap. According to this the picture ranges from those plants with no oxalic acid and a relatively large proportion of sap soluble calcium to those plants with large quantities of oxalic acid and little or no soluble calcium.

Table XIII shows the high positive correlation between insoluble calcium and insoluble oxalates in both the 1940 and 1941 crops.



Fi . 7. Comparison of the amounts of oxalic and citric acid with the amounts of different cations occurring in the plants tested. (1941 crop)

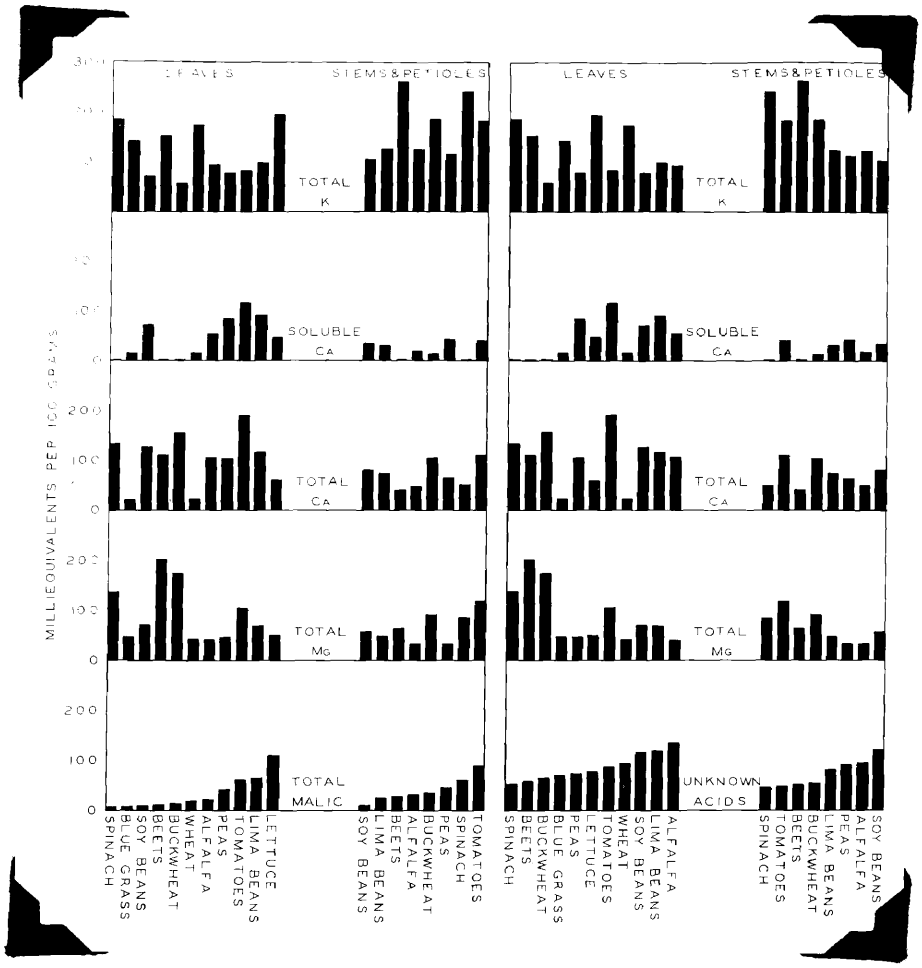


Fig. 8. Comparison of the amounts of malic acid and acids of the unknown group with the amounts of different cations occurring in the plants tested. (1941 crop)

Figure 9 presents the same data in graphic form for the 1941 crop. It will be noted that in the three plants producing the largest quantities of oxalic acid, insoluble oxalates exceed the amount of insoluble calcium. Obviously some other cation besides calcium must exist in the form of an insoluble oxalate in the plant. Whenever juice of beets, spinach, or buckwheat is allowed to stand for a few hours a white crystaline precipitate appears. This has been identified as pure magnesium oxalate. Previous evidence has been obtained by the author (14) that magnesium is precipitated by oxalic acid. Magnesium oxalate is known to form a supersaturated solution rather easily and it appears that this is what happens in the plant. However with an increased uptake of magnesium the limits of supersaturation are bound to be exceeded and some of the magnesium oxalate will be precipitated. In connection with this it should be mentioned that Dunne (6) has presented evidence indicating that in buckwheat oxalic acid can be precipitated as potassium oxalate.

Titratable Acidity and Free Acid Plus Free Phosphate.

Considerable work has been done in trying to identify plant buffers by duplicating buffer curves of plant sap. This duplication is accomplished by mixing substances as KH_2PO_4, various organic acid salts, amino acids, and sugars which are known to occur in plants and which show buffering action at various hydrogen-ion concentrations.

Hurd-Karrer (9) has succeeded in duplicating the buffer curve of the expressed juice of wheat over a wide range of hydrogen-ion concentration. She concluded that for wheat the principal buffers between pH 5.8, 6.0 and 8.0 were phosphate and a small amount of organic acids. Pucher, Vickery, and Wakeman (19) in criticizing the technique of using titratable

TABLE XIII

Correlation between insoluble oxalic acid and insoluble calcium

1940 Crop

Plant	Milliequivalents per 100 grams of dry tissue			
	Leaves		Stems and Petioles	
	Insoluble Oxalic acid	Insoluble calcium	Insoluble Oxalic acid	Insoluble calcium
Lima beans	30.2	36.7	29.6	24.2
Peas	—	—	—	—
Alfalfa	30.0	63.0	9.8	29.3
Soy beans	17.4	56.5	21.8	41.5
Beets	209.8	96.7	37.9	23.1
Spinach	155.0	126.3	84.3	72.3
Buckwheat	242.9	141.8	109.2	92.0
Blue grass	0.0	4.3		
Wheat	0.0	5.6		
Tomatoes	69.3	92.0	82.1	70.5
Lettuce	2.3	32.9		
	Corr. coef. = +.886		Corr. coef. = +.929	

1941 Crop

Lima beans	16.9	27.5	31.1	43.5
Peas	51.1	19.0	7.3	22.9
Alfalfa	14.1	55.1	4.9	31.3
Soy beans	15.9	57.1	18.9	45.0
Beets	161.0	110.7	66.1	39.6
Spinach	167.4	132.6	49.4	48.7
Buckwheat	239.8	154.2	100.6	92.5
Blue grass	0.0.0	6.0		
Wheat	0.0	7.7		
Tomatoes	45.2	74.9	81.3	72.3
Lettuce	1.4	13.2		
	Corr Coef. = +.946		Corr. coef. = +.884	

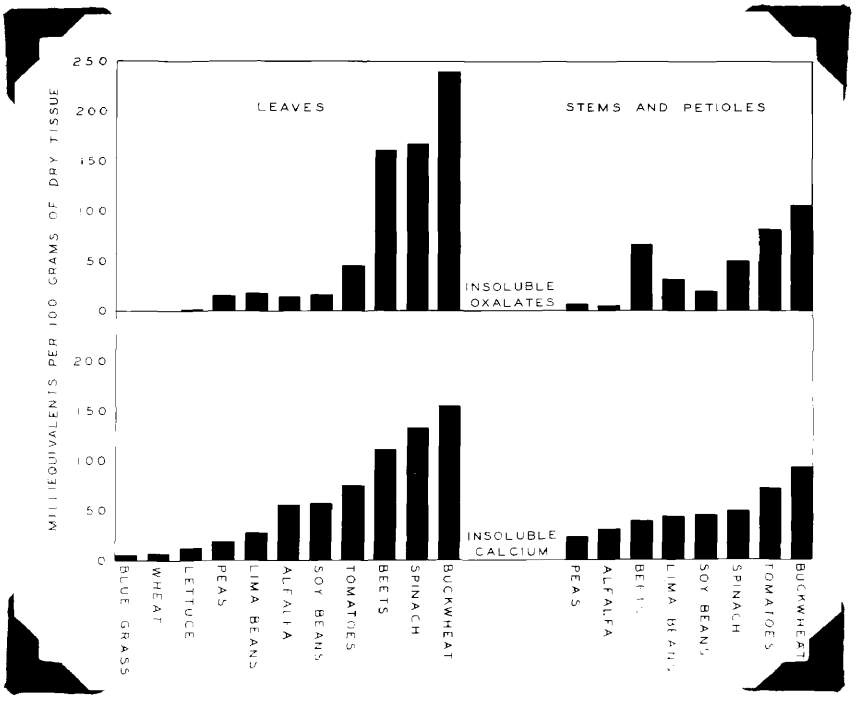


Fig. 9. Relationship between insoluble calcium and insoluble oxalates in leaves and stems of plants tested. (1941 crop).

acidity as a measure of the change in organic acidity state that such a determination is merely a measure of the water soluble acids that remain unneutralized at the pH of the cell sap.

In the present experiment an attempt has been made to determine the main buffers in the plant sap that titrate between the PH of the extracted juice and pH 8.5. This was accomplished by calculating the amount of $H_2PO_4^-$ free at the pH of the sap and adding it to the amount of organic acids free at the same pH. This combined value was then compared with the titratable acidity.

The pH of the sap of the plants tested shows that phosphate can exist only as the $H_2PO_4^-$ and HPO_4^{--} ions. Between the pH limits chosen the entire amount of $H_2PO_4^-$ but none of the HPO_4^{--} is titrated. Therefore the amount of $H_2PO_4^-$ ion present at the pH of the sap was used as the amount contributed by phosphate to the titratable acidity. The total phosphorus content of the plant tissue was used in this calculation rather than just that present in the sap. Even so the data indicate that no great error resulted. In calculating the amount of organic acids free at the pH of the sap the same method was used as for phosphorus, Bennet-Clark (2). Citric and malic acids were considered as entirely soluble while only the soluble portion of the oxalic acid if any was considered. How to calculate the unknown group of acids presented a problem. It was finally decided to consider them all as four carbon acids similar to malic acid and so the dissociation constants of malic acid were used in the calculations. By graphing the amounts of the different ions of the acids existing at various pH values it was possible to read directly the percent of an acid present in any form at a given pH. It was found that all of the commonly occurring organic acids are completely titrated at pH 8.5.

Table XIV shows the high degree of correlation between titratable acidity and free acid plus free phosphate for the 1941 crop. Considering the estimations that it was necessary to make, the equivalence between the two values in many cases is remarkable. It can be seen from figure 10 that the free acid is not correlated with titratable acidity but when free phosphate is added to free acid the correlation is very pronounced. Any large discrepancies as in tomato leaves and buckwheat stems are probably due to the necessity of calculating the unknown acids on the basis of malic acid and using total phosphorus rather than soluble phosphorus in the calculations.

TABLE XIV

Relationship between titratable acidity and free acid plus free phosphate. (1941 crop)

Plant	pH of plant sap	Total free acid	Total free $H_2PO_4^-$	Free acid plus free $H_2PO_4^-$	Titratable acidity
		Leaves			
		Milliequivalents per 100 grams of dry tissue			
Lima beans	5.95	16.5	11.3	27.8	26.4
Peas	5.90	16.8	20.8	37.6	33.7
Alfalfa	6.05	9.2	16.1	25.3	22.0
Soy beans	6.17	11.5	19.2	30.7	23.3
Beets	6.27	5.0	17.9	22.9	18.7
Spinach	6.30	3.3	28.2	31.5	28.1
Buckwheat	5.94	6.8	23.3	30.1	26.8
Blue grass	6.46	3.0	14.5	17.5	20.1
Wheat	5.90	9.0	31.4	40.4	38.6
Tomatoes	5.71	28.1	27.3	55.4	42.0
Lettuce	6.14	10.4	23.1	33.5	31.0
		Corr. coef. +.919			
		Stems and Petioles			
Lima beans	5.68	19.8	14.0	33.8	31.2
Peas	6.01	10.4	12.8	23.2	26.3
Alfalfa	5.85	11.8	19.4	31.2	24.5
Soy beans	5.78	16.3	21.2	37.5	40.1
Beets	5.88	9.2	13.4	22.6	21.4
Spinach	5.95	8.6	32.7	41.3	34.9
Buckwheat	4.69	36.3	21.9	58.2	44.4
Tomatoes	5.55	22.8	27.8	50.6	51.2
		Corr. coef. +902			

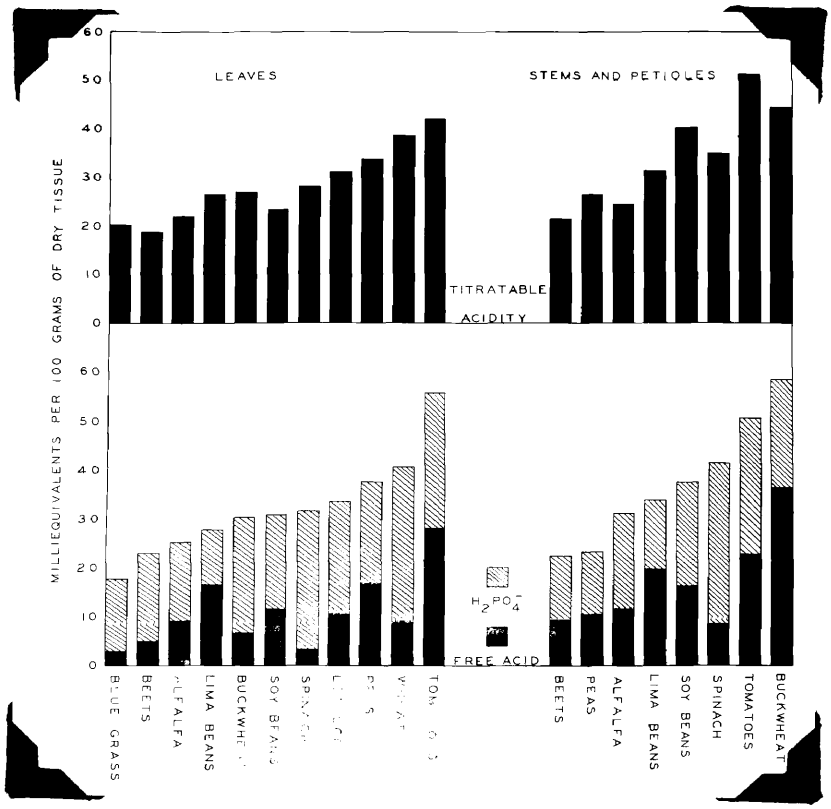


Fig. 10. Relationship between titratable acidity and free acid plus free phosphate in leaves and stems of plants tested. (1941 crop)

Fig. 11. Lima beans (1941)

Fig. 12. Peas (1941)

Fig. 13. Alfalfa (1941)

Fig. 14. Soy beans (1941)

Fig. 15. Beets (1941)

Fig. 16. Spinach (1941)

Fig. 17. Buckwheat (1941)

Fig. 18. Blue grass (1941)

Fig. 19. Wheat (1941)

Fig. 20. Tomatoes (1941)

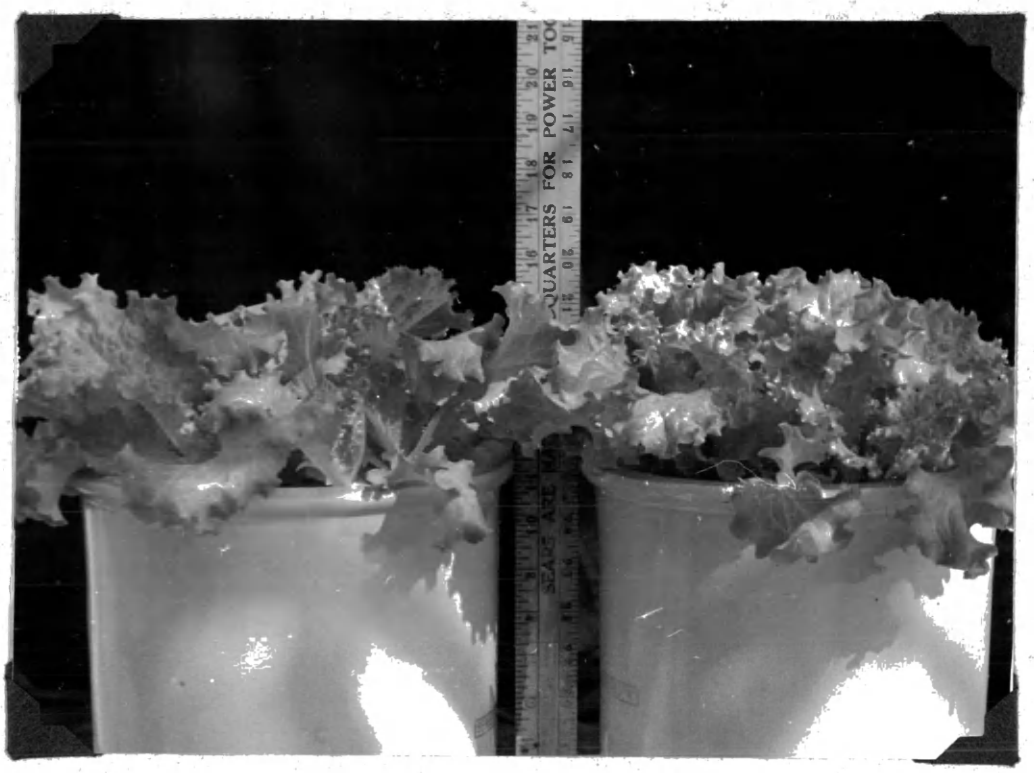


Fig. 21. Lettuce (1941)

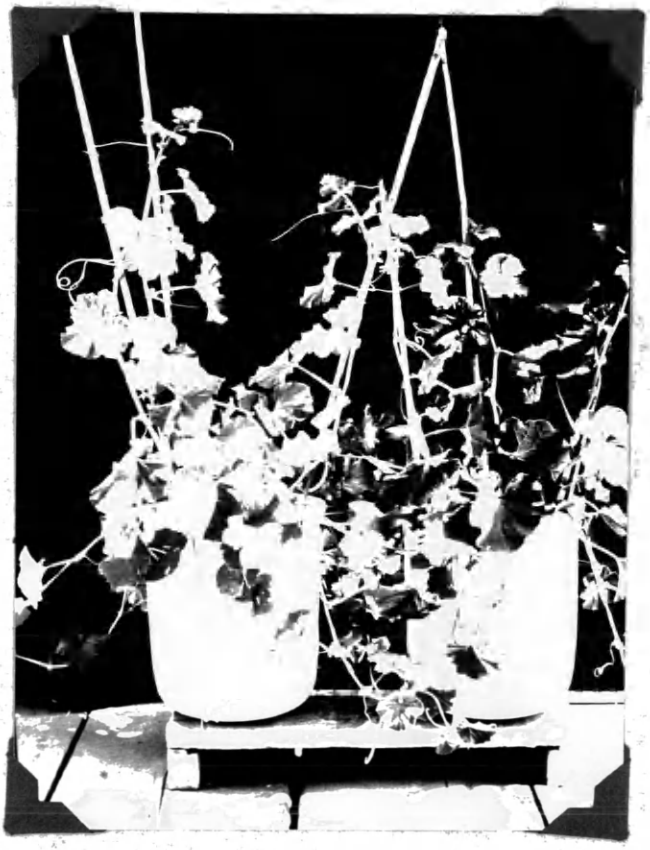


Fig. 22. Cantaloupe (1941)

SUMMARY AND CONCLUSIONS

Twelve different species of plants were grown in the greenhouse under conditions of controlled solution culture and all plants received the same nutrient supply. Chemical analyses were made with the idea of studying the cation-anion balance in the different species of plants.

Inorganic ions were found to be taken up in varying proportions according to inherent characteristics of the plant. Plants in the same family tended to accumulate ions in relatively the same proportion.

Data were obtained on the kinds and amounts of organic acids occurring in a variety of plants. In some plants the unknown fraction of organic acids made up 70 -80 percent of the total while in others the unknown fraction amounted to only 15 - 25 percent.

In all plants a large excess of inorganic cations over inorganic anions was found and when all plants, with the exception of cantaloupe, were considered together this excess was found to be highly correlated with total ether soluble organic acids. Cantaloupe was an outstanding exception. It contained the largest amounts of cations but the smallest amounts of organic acids of any of the plants tested. Obviously organic acids do not play an important role in balancing cations in this plant.

No outstanding example of any one organic acid causing selective absorption of a specific cation was found. In the leaves malic and citric acids showed a rather low positive correlation with soluble calcium. Citric acid and those acids of the unknown group showed a rather small negative correlation with total magnesium content in the stems and petioles.

Insoluble oxalates and insoluble calcium were found to be highly correlated when the plants were considered as a group. In three cases insoluble oxalic acid exceeded the amount of insoluble calcium. Evidence was cited that the additional insoluble oxalic acid was present as magnesium oxalate. Magnesium content increased with increased oxalic acid content.

Those plants with little or no oxalic acid had a large proportion of the calcium in a sap soluble state while those plants high in oxalic acid had but traces of sap soluble calcium.

A comparison of titratable acidity with free phosphate plus free organic acids showed a high degree of positive correlation, in many cases a remarkable equivalence. Free organic acid alone was not correlated with titratable acidity.

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