RÔLE OF ETHER SOLUBLE ORGANIC ACIDS IN THE CATION-ANION BALANCE IN PLANTS[1]

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(WITH SIX FIGURES)

Introduction

Factors that influence salt absorption by plants have long been a subject of study. Only recently, however, (**3, 4, 7, 8, 11, 16, 20, 23**), have the effects of certain metabolic processes in the plant been recognized as intimately connected with salt absorption and accumulation.

The present investigation has been concerned with the cation-anion balance in several species of crop plants with special reference to the rôle of ether soluble organic acids. Prior to the work of PUCHER, VICKERY, and WAKEMAN (**13, 14, 15**), on the quantitative determination of organic acids in plant material, lack of accurate methods had seriously curtailed investigation along these lines. A study has been made of the selective capacity of the various species with respect to inorganic ions; of the quantity and kinds of organic acids in the different species grown in similar culture medium; and of the correlation between inorganic cations and ether soluble organic acids in the different species as a group.

Materials and methods

CULTURE METHODS

Twelve species of plants were chosen for this investigation: spinach, variety Summer Savoy (*Spinacea oleracea* L.); beets, variety Early Superb (*Beta vulgaris* L.); wheat, variety Leapland (*Triticum aestivum* L.); Kentucky blue grass (*Poa pratensis* L.); alfalfa (*Medicago sativa* L.); lima bean (*Phaseolus limensis* Macf.); peas, variety Alaska (*Pisum sativum* L.); soybean, variety Biloxi (*Glycine soja* Sieb. and Zucc.); buckwheat (*Fagopyrum esculentum* Gaertn.); lettuce, variety Grand Rapids (*Lactuca sativa* L.); cantaloupe, variety White Seeded Pink Meat (*Cumcumis melo* L.); and tomato, variety Break of Day (*Lycopersicon esculentum* Mill.). 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 nutrient solution used had the following composition: 0.005 M $Ca(NO_3)_2$, 0.0025 M KH_2PO_4, and 0.0025 M $MgSO_4$. The elements Cu, Mn, Zn, B, and Fe were used in the usual small quantities to supplement the three main salts in the nutrient solution. This solution was applied to all plants at the rate of approximately 1 liter per crock, per 24 hours.

A modification of the SHIVE and STAHL capillary drip method (**19**) was

[1] Scientific Contribution no. 1840, Article no. A27, Department of Botany, University of Maryland Agricultural Experiment Station.

used to apply the nutrient solution. The modification consisted of a series of enamel basins, usually 3, connected by siphons and constantly supplied by solution from an inverted 18-liter bottle. The basins were placed on a shelf between twin rows of coffee urn liners filled with sand; these crocks permit good drainage. The shelf was 2 to 3 inches higher than the tops of the crocks. The basins were covered with round lids cut from Celotex and painted with asphalt paint to prevent water absorption. Four crocks were supplied with nutrient from each basin by inserting capillary tubes into notches cut on the under side of the lids. Adjustment of the capillary tubes was facilitated by adjustable metal supports fastened to the shelf near the basins. The inverted 18-liter bottle maintained a constant head of pressure and insured uniform delivery of nutrient solution at all times. After the initial adjustment of the capillaries, which was done with the aid of a stop-watch, the only thing required was to keep the 18-liter reservoir filled.

The 1940 crop was planted the latter part of September and all plants had been harvested by the end of November. 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. The night temperature of the greenhouse was between 65° and 70° F.

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.

Tissue preparation and analytical methods

All samples were taken between 9:00 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 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 oven at 70° C., ground to pass a 60-mesh sieve, then stored in aluminum boxes until ready for analysis. 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, subjected to 10,000 pounds pressure per square inch in a Carver hydraulic press, and the juice collected. The methods of the Association of Official Agricultural Chemists (1) were used for the final determination of calcium, magnesium, potassium, sulphur, and nitric acid.

MOISTURE.—Immediately after the plants were cut, 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. All results are based on dry tissue.

ASHING.—Duplicate ¾-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 official method (**1**) was used.

PHOSPHORUS AND SULPHUR.—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 magesium nitrate method (**1**). Phosphorus in the ash was determined by the method of FISKE and SUBBAROW (**6**). An Aminco photometer was used for the comparisons. A standardization curve was constructed, using varying concentrations of pure KH_2PO_4. Sulphur in the ash solution was determined by the official method (**1**).

TOTAL NON-VOLATILE ORGANIC ACIDS, OXALIC AND CITRIC ACIDS.—The methods used were those of PUCHER, VICKERY, and WAKEMAN (**13, 14**).

MALIC ACID.—The uranium acetate method of DUNBAR and BACON (**5**), modified by VICKERY and PUCHER (**21**) was used. Admittedly this method is not as good as the one developed later by PUCHER, VICKERY, and WAKEMAN (**15**) but the time saved justified its use.

NITRIC ACID.—The method used for the extraction of the organic acids also extracted the nitric acid quantitatively. A suitable aliquot was reduced, distilled, and Nesslerized (**1**). Readings were taken in an Aminco photometer and the quantity of nitrogen read from a standardization curve.

SAP ANALYSIS

The determination of sap soluble constituents in the extracted juice is based on the work of SAYRE and MORRIS (**17, 18**).

TOTAL SOLIDS.—Duplicate 2-ml. samples of the juice were dried in a hot air oven at 70° C., then placed in a vacuum oven at 65° C. and 2 to 3 cm. pressure until constant weights were obtained.

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 normal 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. From this point the method was identical with that used by PUCHER, VICKERY, and WAKEMAN (**13**) to determine 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

TABLE I

INORGANIC CATIONS, 1940 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE				
		TOTAL MAG-NESIUM	TOTAL POTAS-SIUM	TOTAL CALCIUM	SOLUBLE CALCIUM	SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	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
Soybean	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
Bluegrass	Leaves	52.6	155.1	24.6	20.3	82.6
Wheat	Leaves	52.1	191.5	22.6	17.0	75.3
Tomato	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

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 official micro method (**1**).

Results

The plants from the 1940 and 1941 crops were quite comparable with respect to age, height, and fresh weight, 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 the difference in the amount of growth of the two crops, analyses based on dry weight showed them to be quite comparable. The absolute values for age, height, and fresh weight are not recorded as they were of importance only in comparing the growth of the two crops.

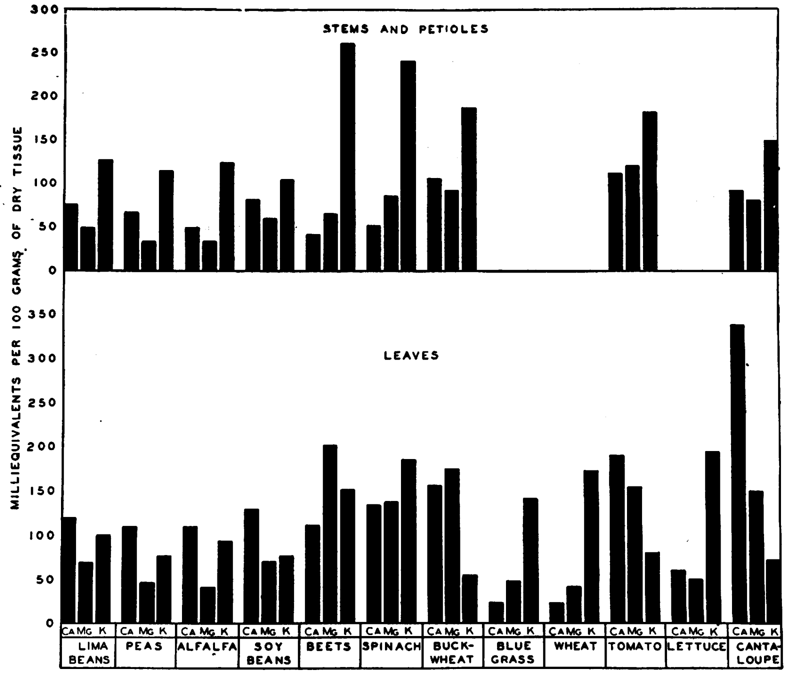

FIG. 1. Distribution of Ca, Mg, and K in leaves and stems of the species tested (1941 crop).

INORGANIC CATIONS AND ANIONS

Table I gives the inorganic cations in milliequivalents per 100 grams of dry tissue in the different plants for the 1940 crop and table II 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 fiigure 1. Figure 2 shows graphically the distribution of calcium. Since the 1940 crop was similar it is not included.

It is clearly shown that when different species of plants are all grown under similar 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 all tend to accumulate cations in the same relative proportions (legumes: soy beans, lima beans, alfalfa, and peas; the Chenopodiaceae: beets and spinach; the grasses: bluegrass and wheat. These results corroborate those of NEWTON (9).

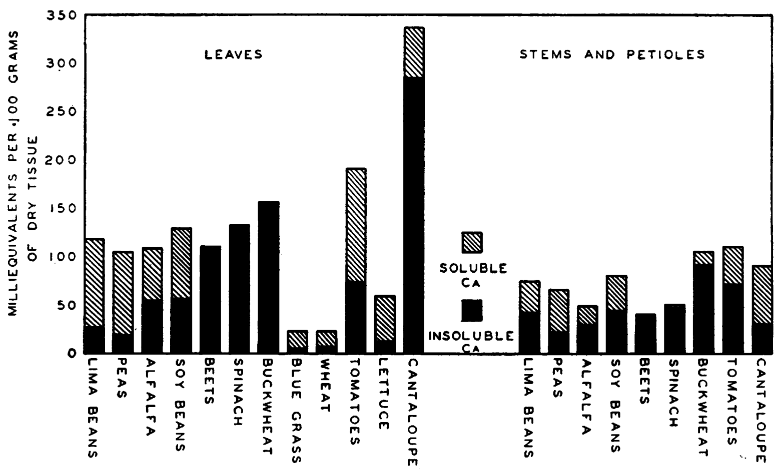

FIG. 2. Relative amounts of soluble and insoluble calcium in leaves and stems of the species tested (1941 crop).

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 potas-

TABLE II

INORGANIC ANIONS AND CATIONS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE							SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		TOTAL NO^-_3	TOTAL $SO^=_4$	TOTAL H_2PO_4	TOTAL MAGNESIUM	TOTAL POTASSIUM	TOTAL CALCIUM	SOLUBLE CALCIUM	
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	28.4	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
Soybean	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	201.8	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
Buckwheat	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
Bluegrass	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	173.1	22.7	15.0	66.7
Tomato	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

sium. Much larger quantities of this element accumulate 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 percentage of the total ranges from 70 to 80 per cent. in some plants and down to 1 per cent. in others.

Organic acids

Tables III and IV give the values of the various organic acids in milliequivalents per 100 grams of dry tissue in the two crops, 1940 and 1941.

TABLE III

Organic acids, 1940 crop

Plant		Milliequivalents per 100 grams of dry tissue: Total acids	Oxalic	Malic	Citric	Unknown acids	Unknown as percentage of total
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	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
Soybean	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
Bluegrass	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
Tomato	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

A word of warning is necessary when comparing the data in this experiment with data from plants grown under different conditions. As Clark (**4**) and Wadleigh and Shive (**23**) have shown and as Vickery and Pucher (**22**) 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 to 80 per cent. of the total; in beets, spinach, and buckwheat 70 to 85 per cent. 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 suitable methods for the determination of the acids of the unknown group.

CATION-ANION BALANCE

PUCHER, VICKERY, and WAKEMAN (**16**) have shown in tobacco grown under controlled fertilizer conditions a large excess of positive ions over inorganic anions, and this excess is balanced by 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.

TABLE IV

ORGANIC ACIDS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE					UNKNOWN AS PERCENTAGE OF TOTAL
		TOTAL ACIDS	OXALIC	MALIC	CITRIC	UNKNOWN ACIDS	
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	249.7	16.9	65.8	48.6	118.4	47.4
	Stems	171.1	31.1	24.5	33.1	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
Soybean	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
Bluegrass	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
Tomato	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

In the analyses no attempt was made to fractionate organic and inorganic sulphur and phosphorus. In the calculations of milliequivalents of anions all sulphur was considered as sulphate and all phosphorus as diacid phosphate. This is not strictly true, as phosphorus and sulphur are known to enter into many organic compounds as proteins, phosphatides, hexose phosphates, etc. It seems safe to assume, however, that the major portions of these elements are in the inorganic form in the plant. All phosphorus was calculated as the monophosphate 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 V presents data showing the relationship between the excess inorganic cations and ether soluble organic acids in the leaves and stems. Figure 3 presents the data of table V in graphic form.

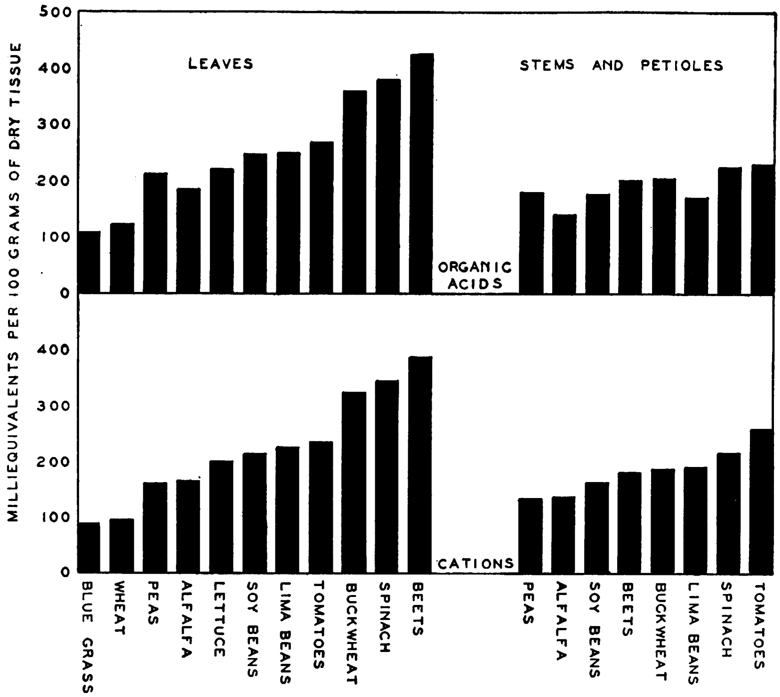

FIG. 3. Correlation between excess cations and total organic acids in leaves and stems of several species (1941 crop).

As far as the authors are aware these are the first data to show that inorganic cations and ether soluble organic acids are positively correlated in

TABLE V

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 ANIONS*	EXCESS CATIONS†	TOTAL ORGANIC ACIDS	TOTAL Ca, Mg, K	TOTAL ANIONS*	EXCESS CATIONS†	TOTAL ORGANIC ACIDS
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Lima bean	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
Soybean	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
Buckwheat	389.7	65.8	323.9	360.1	384.8	196.8	188.0	205.6
Bluegrass	212.9	124.3	88.6	108.5	......	......	......	......
Wheat	237.0	142.6	94.7	122.1	......	......	......	......
Tomato	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	......	......	......	......
Cantaloupe	557.9	143.6	414.3	99.2	320.8	174.2	146.6	119.4

* NO_3^-, $SO_4^=$, $H_2PO_4^-$.
† Total cations minus inorganic anions.

a series of different species of plants. The correlation in the leaf blade tissue is exceedingly high (+ 0.996), that in the stem and petiole tissue not as high (+ 0.798).

It will be noted in table V, that the cantaloupe plant has the highest cation content, but the lowest organic acid content, of any of the species tested. The exceedingly high amount of calcium present in the leaves of cantaloupe is rather unusual. Approximately 85 per cent. is insoluble but

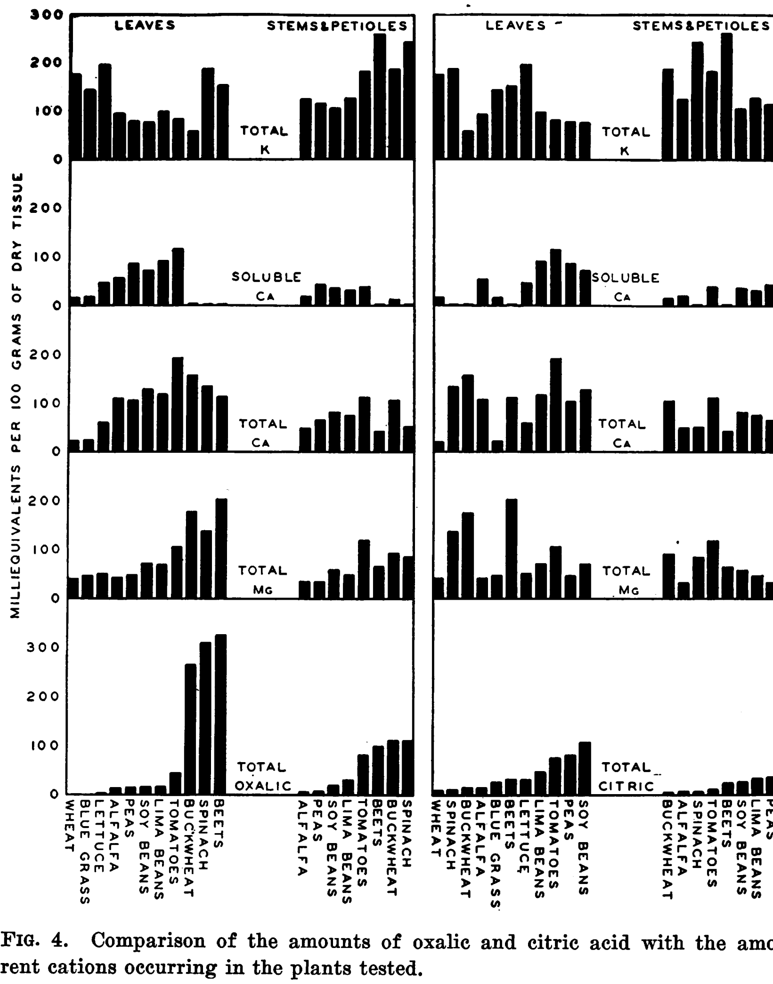

FIG. 4. Comparison of the amounts of oxalic and citric acid with the amounts of different cations occurring in the plants tested.

it is not obvious in what combination it exists. There is no oxalic acid in the cantaloupe plant so the possibility of insoluble calcium oxalate is excluded. The pH 7.4 to 7.6 of the expressed sap is unusually high. This immediately suggests the possibility that part of the cations are tied up as carbonate and 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, sulphate, nitrate, and organic acids are binding the large quantities of cations in this plant. ILJIN (8) found, in general, that soluble calcium in plants was positively correlated with malic and citric acid content. PIATNITSKY (11) found that excised leaves of adult tobacco plants accumulated citric acid when exposed to a solution of $MgCl_2$. The increase in citric acid was nearly equivalent to the decrease in malic acid.

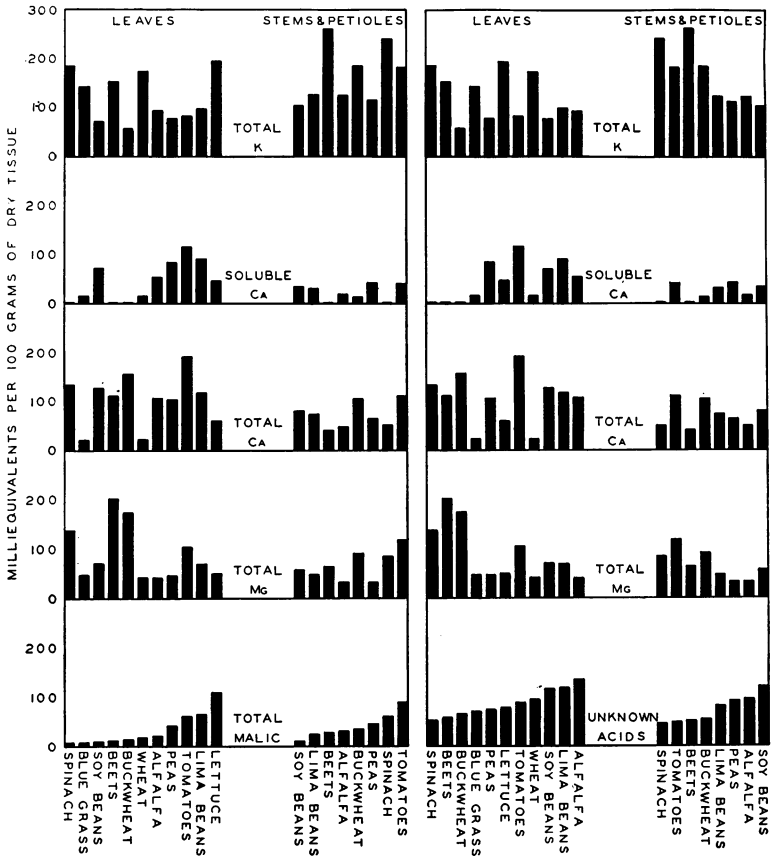

FIG. 5. Comparison of the amounts of malic acid and acids of the unknown group with the amounts of different cations occurring in the plants tested (1941 crop).

Figures 4 and 5 compare graphically the amounts of determined organic acids as well as the unknown group with the amounts of cations found in the various plants. No striking correlations 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.

In spinach, beets, and buckwheat a relatively large proportion of the organic acids could be accounted for principally as oxalic acid. Figure 4 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.

Results of the present investigation indicate that those plants which produce no oxalic acid have very small quantities of insoluble calcium (figures 4 and 6). 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. The major portion of the calcium, however, is

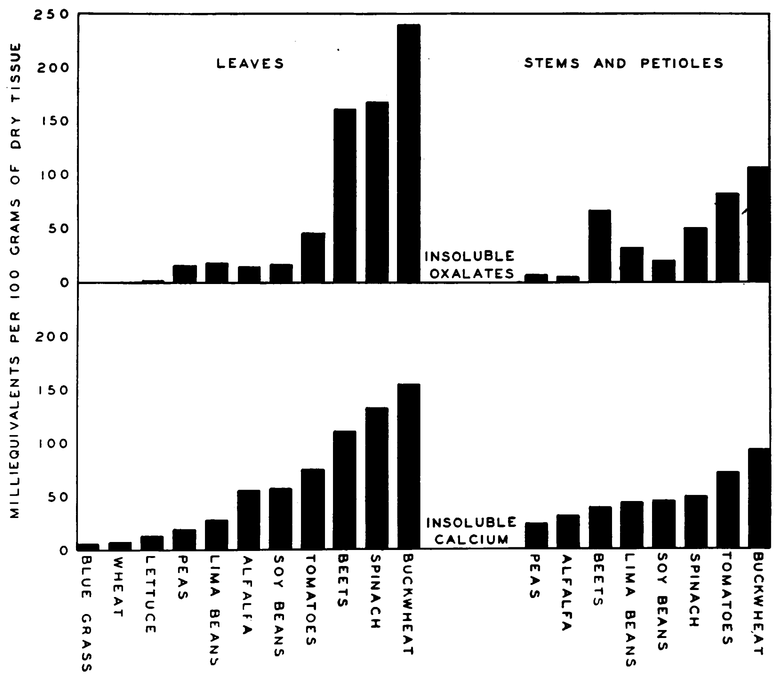

FIG. 6. Relationship between insoluble calcium and insoluble oxalates in leaves and stems of several species tested (1941 crop).

still in a sap soluble state. Those plants (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 VI shows the high positive correlation between insoluble calcium and insoluble oxalates. Figure 6 presents the same data in graphic form for the 1941 crop. It will be noted that in the three plants producing the larg-

TABLE VI

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
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*
Lima bean	30.2	36.7	29.6	24.2
Peas	......	......	......	......
Alfalfa	30.0	63.0	9.8	29.3
Soybean	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
Bluegrass	0.0	4.3	......	......
Wheat	0.0	5.6	......	......
Tomato	69.3	92.0	82.1	70.5
Lettuce	2.3	32.9	......	......
	Correlation coefficient = + .886		Correlation coefficient = + .929	
1941 CROP				
Lima bean	16.9	27.5	31.1	43.5
Peas	51.1	19.0	7.5	22.9
Alfalfa	14.1	55.1	4.9	31.3
Soybean	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
Bluegrass	0.0	6.0	......	......
Wheat	0.0	7.7	......	......
Tomato	45.2	74.9	81.3	72.3
Lettuce	1.4	13.2	......	......
	Correlation coefficient = + .946		Correlation coefficient = + .884	

est 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 crystalline precipitate appears. This has been identified as pure magnesium oxalate. Previous evidence has been obtained by the senior author (**12**) 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. With an increased uptake of magnesium, however, the limits of supersaturation are bound to be exceeded and some of the magnesium oxalate will be precipitated.

Summary and conclusions

Twelve different species of plants were grown in the greenhouse under 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 proportions.

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 to 80 per cent. of the total, while in others the unknown fraction amounted to only 15 to 25 per cent.

In all of the plants studied, except cantaloupe, a large excess of inorganic cations over inorganic anions was found and when all plants were considered together this excess was found to be highly correlated with total ether soluble organic acids. Cantaloupe was the outstanding exception. It contained the largest amounts of cations but the smallest amounts of organic acids of any of the species tested.

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.

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