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† POTASSIUM DEFICIENCY AND EXCESS IN GUAYULE.
II. CATION-ANION BALANCE IN THE LEAVES †

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(WITH ONE FIGURE)

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In many plants the potassium contained in the tissues is almost completely water soluble (3, 15, 16, 18, 19, 32), although this is not invariably the case (8). Potassium is frequently the chief soluble mineral cation present in the cellular fluids, particularly in plants which normally contain little calcium, such as wheat and bluegrass, and in those in which most of the calcium is present in insoluble forms such as spinach and buckwheat (8, 21). As emphasized by HOAGLAND (13) with particular reference to plants deficient in potassium a decrease in fixed base in the sap may result if much of the calcium and magnesium goes out of solution in the plant, even though the content of total calcium and magnesium in the tissues as a whole increases by amounts equivalent to the decreased absorption of potassium.

It is generally supposed that much of the potassium present in plants is in the form of salts of organic acids and that these acids and their salts constitute principal components of the buffer systems. A relationship between the total organic acids and the excess of cations over inorganic anions in tobacco leaves of different ages and base composition was shown by PUCHER, VICKERY, and WAKEMAN (22). PIERCE and APPLEMAN (21) grew several species of plants in the same nutrient medium and showed a general correlation between the excess of cations and the total organic acids. In these correlations, however, were included both soluble and insoluble constituents, and variations in insoluble oxalate and calcium contributed much to the variations in the total bases and organic acids. ULRICH (33) has shown that the total organic acid content of sap expressed from barley roots may be varied by unequal absorption of cations and anions from the solution. An absorption of cations in excess of anions results in an in-

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crease in organic acids. By employing calcium bromide, the organic acid content of the roots can be shown to decrease when bromide ions are absorbed in excess of calcium ions.

Effects of different rates of potassium supply upon the growth and mineral contents of guayule plants have been reported in a previous publication (5). It was noted that although the nutrient treatments resulted in large differences in concentrations of potassium and other cations within the plants, the total milliequivalents of cations per 100 grams of dry tissue for the whole plants was nearly the same for all treatments. In this paper are reported results from a study of the cation-anion balance in the leaves of the guayule plants previously described (5). An attempt was made to determine the effects of variations in contents of potassium and concomitant variations in contents of other cations upon the distribution of ions between soluble and insoluble fractions.

Materials and methods

The culture methods employed in growing the experimental plants have been described (5). Treatments designated K_1 , K_2 , and K_3 comprised series 1. The nutrient solutions for these treatments contained 0.5, 3, and 12 milliequivalents K per liter, respectively. These solutions each contained 10 m.e. Ca per liter and no Na except as impurities. Treatments designated NaK_1 , NaK_2 , and NaK_3 comprised series 2. The solutions supplied to these plants contained the same concentrations of K as the corresponding solutions of series 1, but each solution of series 2 contained 4 m.e. Ca and 6 m.e. Na per liter.

At final harvest 100 grams of fresh tissue from each leaf sample were tightly sealed in one quart fruit jars. The jars were packed in dry ice and stored there until immediately preceding analysis (at least 48 hours). Rapid and carefully controlled thawing was found to be essential to obtain reproducible estimations of the carbonate components. To this end, the frozen sample was rapidly transferred to a two quart jar. The jar was then sealed and rotated in water at 50° C. for 15 minutes. At this point the material was completely thawed but still cold. The sample was then placed in a muslin cloth and subjected to 1300 pounds pressure per square inch (effective pressure at the surface of the tissue mass) in a Carver hydraulic press. This pressure was maintained until flow of the sap had practically ceased. The pressing operation required six to seven minutes.

For total solids, five ml. samples of expressed sap were evaporated to about 1 ml. on a water bath and dried to constant weight in a vacuum oven at 65° C. and 3 to 4 cm. pressure. For specific gravity, five-ml. aliquots of fresh sap were weighed in weighing bottles of 5 ml. capacity. The sample used for determining total solids was ashed by the magnesium nitrate method (1). Sulphur in the ash solution was determined by the official method (1). A separate aliquot was employed for the determination of phosphorus by the method of FISKE and SUBBAROW (9).

For calcium and magnesium, five ml. aliquots of sap were taken to dryness on a steam bath, moistened with sulphuric acid (1-10), dried in an oven at 105° C. and ashed at 600° C. for 16 hours. The ash was dissolved in dilute HCl, taken to dryness to dehydrate any silica, redissolved in HCl. Calcium and magnesium in the ash solutions were determined by the official method (1). Potassium was determined by the method of HIBBARD and STOUT (12). Nitrates were determined by the phenoldisulfonic method (10) as described by SCHLENKER (28).

For total non-volatile organic acids, malic acid, and citric acid, 10 ml. aliquots were concentrated to 1-2 ml. in a vacuum oven at 50° C. and 3-4 cm. pressure. The ether extraction was performed in a Goldfish extraction apparatus. Twelve-hour extraction periods were employed. A Beckman pH meter was used for titration of the ether extracts. The procedures were otherwise identical to those described by PUCHER, VICKERY and WAKEMAN (23, 24), and PUCHER, VICKERY and LEAVENWORTH (25).

Bicarbonate was estimated from the carbon dioxide which could be aspirated from the sap after acidification with hydrochloric acid containing stannous chloride. The procedure used was essentially the official method for determination of carbonate in soils (1). In place of the sample flask, a 175 mm. × 22 mm. test tube was used. A stream of carbon dioxide-free air was introduced at the bottom of the test tube through a Folin bulb. The samples were aspirated slowly for two hours into the absorption towers. Five ml. of sap were introduced into the test tube immediately after expression; 1.3 ml. of 33 per cent. SnCl₂ in HCl (1-1) were added rapidly and aspiration begun. Blank determinations were run with each series. Quantitative recovery of carbon dioxide was obtained from standard solutions of sodium carbonate.

For the analysis of press cakes, they were weighed immediately after expression of the sap, then dried to constant weight in a vacuum oven at 65° C. and 3-4 cm. pressure. The dried press cakes were ground to a fine powder in a micro Wiley mill. One-half gram samples were used for the determination of calcium, magnesium, sulphur, and phosphorus. The procedures were the same as those employed for the dried sap. Total non-volatile organic acids in the press cakes were determined as in the sap. 2.00 gram portions were used for this purpose. Individual acids were not determined in view of the relatively low results obtained from all samples. The phosphorus contents of the ether extracts were determined and corrections applied as described by PUCHER, VICKERY and LEAVENWORTH (25). Carbonate in the press cakes was determined essentially as was the bicarbonate content of the sap. Eight ml. of 10 per cent. SnCl₂ dissolved in HCl (1-9) were used for acidification of 0.5-gram samples. During the aspiration the tubes were shaken until the sample was thoroughly wetted.

Sodium was determined in the dried leaves samples (5). In some cases, dried samples were also employed for determination of potassium.

CALCULATION AND EXPRESSION OF RESULTS

Estimation of sap soluble constituents was based on the work of SAYRE and MORRIS (26, 27) for estimates of total constituents (i.e., soluble + insoluble). The contributions from the sap and from the press cake were calculated from the proportions of the total fresh weight represented by sap and press cake respectively. Comparable results could be obtained from dried whole leaf samples as is shown by a comparison of potassium contents of the whole leaves based on determination of potassium in the sap and press cakes, or in whole leaf samples (table I).

TABLE I

POTASSIUM CONTENT OF MATURE LEAF TISSUE BASED ON COMPOSITIONS OF SAP AND PRESS CAKE AND ON COMPOSITION OF DRIED WHOLE LEAF SAMPLES

TREATMENT	MILLIEQUIVALENTS PER KILOGRAM OF FRESH TISSUE			
	TOTAL K			SOLUBLE K
	SAP PLUS PRESS CAKE	WHOLE DRIED SAMPLE	AVERAGE	
K ₁	73	71	72	65
K ₂	247	243	245	233
K ₃	429	427	428	423
NaK ₁	119	125	122	116
NaK ₂	289	287	288	288
NaK ₃	447	457	452	448
Average	267.0	268.3	262.2

A comparison of soluble and total potassium is also shown in table I. A very small insoluble fraction is indicated, in agreement with results recorded for tissues of other plants (19, 20). However, in this case the insoluble fraction approaches so nearly the magnitude of the accuracy with which the determinations could be made, that a separate account of it does not seem appropriate. Accordingly potassium has been expressed as entirely soluble. The relatively small quantities of sodium found in the leaves have also been considered as soluble.

Samples from each of the eighteen plots (i.e., 3 replicates of each treatment) were analyzed separately. Analysis of variance (30) was applied to the analytical data.

Results

RECOVERY OF CARBONATE FROM THE LEAVES

It was evident from preliminary observations that quantities of the components of the carbonic acid system were present in guayule leaves and that this system would require consideration in the cation-anion balance of the leaves. SMALL (29) has pointed out that in all experiments with expressed sap the escape of carbon dioxide will be a source of error, especially where a bicarbonate system is present, as the bicarbonate-carbonic

acid ratio in the uninjured cell may differ from that which will result when the carbon dioxide content forms an equilibrium with the air. In the present study the primary object was to obtain sufficiently complete recovery of the various cations and anions present. A second consideration was the fractionation of soluble and insoluble components. The question was, whether by analysis of the expressed sap and of the press cakes a satisfactory separation of soluble and insoluble components of the carbonate system could be obtained without too great an error resulting from loss of carbon dioxide. Accordingly experiments were devised to determine the recovery which could be obtained by various procedures.

A 500-gram sample of young (expanding) leaves was gathered from 24

TABLE II

EFFECTS OF PRETREATMENT UPON RECOVERY OF CARBONATE AND UPON DISTRIBUTION OF CARBONATE AND PHOSPHATE BETWEEN SOLUBLE AND INSOLUBLE FRACTIONS

DESIGNATION AND PRETREATMENT	MILLIMOLES PER KILOGRAM OF FRESH LEAF TISSUE						pH OF SAP OR EX- TRACT
	CARBONATE			PHOSPHATE			
	SAP SOLU- BLE	IN- SOL- UBLE	To- TAL	SAP SOLU- BLE	IN- SOL- UBLE	To- TAL	
A ₁ . Frozen in "dry ice," thawed 15 min. at 50° C.	14 ± 0.9	218 ± 5.4	232 ± 5.2	9.2	20.6	29.8	6.66
A ₂ . Frozen in "dry ice," thawed 2 hrs. at room temperature	38 ± 3.8	183 ± 0.3	221 ± 4.2	4.4	26.5	30.9	7.10
A ₃ . Frozen in "dry ice," thawed 7 hrs. at room temperature	58 ± 3.5	165 ± 5.6	223 ± 5.5	1.5	28.3	29.8	7.42
B. Frozen in brine at ca. -20° C.; thawed 5 min. at 50° C.	5 ± 0.7	231 ± 5.2	236 ± 5.8	7.4	24.6	32.0	6.57
WF. Fresh leaf tissue	249 ± 3.0	7.77- 8.20

plants which had received a complete nutrient solution for four months. The sample was thoroughly mixed and triplicate aliquots were employed for each of the treatments indicated in table II. The samples to be frozen were packed loosely in pint fruit jars and tightly sealed. After thawing the tissue and expressing the sap, the pH was determined and 5-ml. portions were taken immediately for determination of carbonic acid.

A 4-ml. aliquot from each sample was combined with other replicates receiving the same treatment. This mixture was used for determination of specific gravity and phosphorus content. The press cakes were handled in the same manner as in the main experiment. The ground fresh samples (WF) were rinsed into the reaction tubes with freshly boiled and cooled distilled water and made to a final volume of about 20 ml. Four ml. of

HCl (1-1) containing 33 per cent. SnCl_2 were added for liberation of carbon dioxide.

Results from determinations of pH, total carbonate and phosphate as well as distribution between soluble and insoluble fractions are shown in table II. Carbonate and phosphate components are expressed as millimoles per kilogram of fresh material. Recovery of total carbonate was highest from unfrozen samples, where fractionation was not attempted (WF). It is evident that the concentration of carbonate components in the sap increased with increasing time of thawing. These increases coincided with decreases in the insoluble fraction. Components of the phosphate system were much lower in concentration than carbonate components, but a decreasing concentration of phosphorus in the sap coincided with increasing amounts of insoluble phosphorus when thawing time was increased. The pH of the sap increased as the time of thawing was prolonged.

Where fresh leaf samples were ground with distilled water, pH values of the dilute suspensions were higher than any of those found in the expressed sap. The relatively high pH values of these suspensions may be explained as an effect of dilution. From results to be presented later (tables IV, V, and VII) it is evident that carbonate was the principal insoluble anion present in the leaves. Dilution of the tissue fluids, therefore, would result in solution of increased quantities of carbonate, but would not appreciably alter the quantities of other anions in solution. Thus, uncombined acids originally present in the tissue fluids would tend to be neutralized. At pH 7.8 at least 96 per cent. of any carbonic acid in solution would be in the form of bicarbonate. It is not likely that any detectable loss of carbon dioxide would be encountered, within the short time required for grinding the tissue and transferring it to the reaction flask. In fact, in these preparations it seems likely that most of the carbon dioxide which was present in the free form in the fresh tissue was recovered. To this extent, this procedure may yield high estimates of the ionic forms of the carbonate system.

In a search for the locus of the insoluble carbonate within the leaf, fresh transverse sections were examined but no inclusions of carbonate could be found in the chlorenchymous tissues. However, strips of epidermis mounted in water gave rise to a considerable evolution of gas when treated with dilute acid. Bubbles could be seen coming from within the epidermal trichomes. No such evolution could be observed from the chlorenchymatous cells when transverse sections were similarly treated with dilute acid. In interpreting the results reported in table II, it must be considered that the insoluble carbonate is localized largely in the epidermal trichomes. When the protoplasm of the leaf cells is disorganized by freezing and thawing, the insoluble carbonate is exposed to an admixture of the tissue fluids. Solution and neutralization of carbonate takes place, and precipitation of phosphate results as the pH of the sap is increased. Although these effects cannot be eliminated completely where the whole leaves are extracted or

pressed, they are minimized by rapid thawing and pressing. Recovery of total carbonate was not impaired seriously where thawing was rapid. The freezing procedure avoids dilution effects attending extraction of the fresh leaves by grinding.

RESULTS FROM THE MAIN EXPERIMENT

In the work of PUCHER, VICKERY and WAKEMAN (22), as well as in that of PIERCE and APPLEMAN (21), approximations have been employed in estimating some of the constituents. Thus the calcium, magnesium, sulphur and phosphorus are certainly not entirely in the ionic forms indicated. For instance portions of the insoluble calcium must be presumed to be in the form of pectates and other organic combinations not taken into account. Likewise some of the insoluble magnesium is certainly within the chlorophyll molecule. A portion of the sulphur is incorporated in the protein and amino acid molecules. In the tobacco leaf tissues employed by Pucher and Vickery, approximately 70 per cent. of the total sulphur was found to be in the form of sulphate, whereas Pierce and Appleman expressed their total sulphur values as sulphate. In the present study, the latter procedure is adopted with respect to sulphur, as no estimate of the proportion of organic sulphur is available for guayule leaves. In the previous studies (*loc. cit.*) total phosphorus has been expressed as dihydrogen phosphate, as was justified by the reactions of the tissues employed, with the assumption that the phosphorus in organic forms such as phosphoproteins, phospholipids, glyco-phosphates, etc. is quantitatively negligible.

In guayule leaves, the relatively high pH values and the presence of the carbonate system introduce additional errors, particularly involving the fractionation into soluble and insoluble components as noted above. Although an attempt was made to minimize these errors by employing a minimum uniform time for thawing and pressing operations, it seems improbable that soluble and insoluble fractions calculated from the concentrations in the expressed sap and press cakes represent soluble and insoluble amounts present in the living leaves with complete accuracy. It is necessary first to present the soluble and insoluble components found representing the phosphate and carbonate in the forms in which they could occur at the observed reactions of the expressed sap. The errors introduced during preparation of the material will then be considered in interpreting these results. Accordingly proportions of soluble monovalent and divalent phosphate are estimated from the observed pH values by use of the Brönstead-Hasselback equation employing the dissociation exponent $pK_2 = 7.20$ (2). This procedure is justified on the basis that the proportions of monovalent and divalent forms differ considerably in the range of reactions observed. In any case, it will be noted (tables IV and V) that the quantity of phosphate in the soluble phase was not of an order of magnitude which would influence the total soluble anion values significantly.

With respect to the carbonate system, the total carbon dioxide evolved from the expressed sap after acidification has been calculated as bicarbonate which is the dominant form in the observed pH range. In this case, too, it may be noted that the magnitude of these values is not great relative to other constituents and that in no case can the resultant totals be very significantly altered by this procedure for estimating bicarbonate. Ammonium ion concentrations were so low in the guayule leaves (6) that an account of them is unnecessary for the balance sheets.

Insoluble carbonate and phosphate are expressed as divalent ions. This seems justified by the low solubilities of the calcium salts of these acids as well as by the observation that aqueous suspensions of the press cakes had reactions of approximately pH 8.5 for all types of leaf samples. In cases where press cakes were farther extracted, suspensions of still more alkaline reaction were obtained. With respect to phosphorus these estimates are high to the extent of phosphorus compounds other than inorganic phosphate.

TABLE III
REACTION OF THE EXPRESSED SAP

TREATMENT	MATURE LEAVES		EXPANDING LEAVES	
	(H ⁺) × 10 ⁷	pH	(H ⁺) × 10 ⁷	pH
K ₁	2.13	6.67	1.44	6.84
K ₂	0.72	7.14	0.63	7.20
K ₃	0.44	7.36	0.31	7.51
NaK ₁	1.16	6.94	0.78	7.11
NaK ₂	0.46	7.34	0.36	7.44
NaK ₃	0.26	7.58	0.28	7.55
DRFS* 0.05	0.67	0.50
0.01	0.87	0.70

* Difference required for significance.

The reactions of the expressed sap are shown in table III. The observed pH values were converted to hydrogen ion concentrations of which the mean values are shown. The mean pH values reported were derived from these mean hydrogen ion concentrations. The pH values from low potassium leaves were consistently lower than those from other treatments in each series and in each age group of leaf samples. The high potassium leaves yielded average pH values consistently higher than those from other treatments, but these differences were not significant statistically. At each level of potassium supply, treatments of series 2 yielded higher pH values than did those of series 1 in each age group of leaves. The pH of sap expressed from expanding leaves was usually higher than that from mature leaves of the same treatment.

In tables IV and V individual and total inorganic cations and anions are partitioned into soluble and insoluble components of mature leaves and expanding leaves respectively. All constituents are expressed as milliequiva-

TABLE IV
INORGANIC CATION AND ANION COMPOSITION OF MATURE LEAVES

TREATMENT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE										MILLIEQUIVALENTS PER KILOGRAM OF FRESH TISSUE	
	K ⁺	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	TOTAL CATIONS	H ₂ PO ₄ ⁻	HPO ₄ ⁼	SO ₄ ⁼	NO ₃ ⁻	TOTAL INORGANIC ANIONS	TOTAL CATIONS	TOTAL INORGANIC ANIONS
SAP SOLUBLE												
K ₁	36	2	141	79	258	1.4	0.9	75	44	121	516	242
K ₂	138	1	81	56	276	0.8	1.3	94	60	156	492	278
K ₃	236	0	38	27	321	0.8	2.3	136	62	201	536	336
NaK ₁	65	16	74	108	263	1.5	1.7	90	39	132	495	248
NaK ₂	162	17	43	73	295	0.8	2.2	109	50	162	525	288
NaK ₃	273	10	26	35	344	0.7	3.2	132	58	194	569	320
DRFS*												
0.05	6.6	3.8	12.1	6.8	18.1	0.35	0.55	11.5	10.7	21.2	34.1	18.1
0.01	9.4	6.3	17.2	9.6	25.7	0.50	0.78	16.3	15.2	30.2	48.4	25.8
INSOLUBLE												
K ₁	218	17	235	14	57	71	471	142
K ₂	208	17	225	11	56	67	401	119
K ₃	124	15	139	8	40	48	232	80
NaK ₁	190	31	221	14	60	74	416	139
NaK ₂	170	26	196	11	54	65	349	116
NaK ₃	90	19	109	10	44	54	180	89
DRFS*												
0.05	19.7	7.1	23.7	1.4	8.1	8.5	38.1	18.8
0.01	27.6	10.0	33.8	2.0	11.5	12.1	54.1	26.7
TOTAL												
K ₁	36	2	359	96	493	1.4	15	132	44	192	987	384
K ₂	138	1	289	73	501	0.8	12	150	60	223	893	397
K ₃	236	0	162	42	460	0.8	10	176	62	249	768	416
NaK ₁	65	16	264	139	484	1.5	15	150	39	206	911	387
NaK ₂	162	17	213	99	491	0.8	13	163	50	227	874	404
NaK ₃	273	10	116	54	453	0.7	13	176	58	248	749	410
DRFS*												
0.05	6.6	3.8	21.5	7.4	31.4	0.35	2.5	12.9	10.7	21.9	46.6	n.s.
0.01	9.4	6.3	30.6	10.5	44.7	0.50	3.6	18.4	15.2	31.1	66.3

* DRFS = Difference required for significance.

TABLE V
INORGANIC CATION AND ANION COMPOSITION OF EXPANDING LEAVES

TREATMENT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE										MILLIEQUIVALENTS PER KILOGRAM OF FRESH TISSUE	
	K ⁺	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	TOTAL CATIONS	H ₂ PO ₄ ⁻	HPO ₄ ⁼	SO ₄ ⁼	NO ₃ ⁻	TOTAL INORGANIC ANIONS	TOTAL CATIONS	TOTAL INORGANIC ANIONS
SAP SOLUBLE												
K ₁	38	1	98	68	205	0.9	0.8	65	51	118	383	220
K ₂	106	1	53	46	206	0.7	1.4	72	55	129	386	242
K ₃	230	2	25	282	230	0.9	3.8	113	67	185	483	317
NaK ₁	69	10	59	85	223	1.5	2.4	75	51	130	402	235
NaK ₂	121	13	35	61	230	0.8	85	85	53	141	425	261
NaK ₃	228	9	26	35	298	1.1	5.0	116	69	191	490	314
DRFS*												
0.05	14.8	1.8	10.5	11.4	25.9	0.37	0.91	12.8	8.5	17.8	27.7	24.0
0.01	21.1	2.9	15.0	16.1	36.8	0.52	1.30	18.2	12.0	25.6	39.3	34.1
INSOLUBLE												
K ₁	191	16	207	17	55	72	386	134
K ₂	177	23	200	15	43	58	375	109
K ₃	97	19	116	11	30	41	199	70
NaK ₁	147	28	175	15	44	59	316	107
NaK ₂	138	23	161	14	39	53	298	98
NaK ₃	74	16	90	9	21	30	148	49
DRFS*												
0.05	11.5	6.3	12.1	3.3	9.9	11.5	27.7	19.1
0.01	16.3	9.0	17.2	4.7	14.0	16.4	39.4	27.2
TOTAL												
K ₁	38	1	289	84	412	0.9	18	120	51	190	769	354
K ₂	106	1	230	69	406	0.7	16	115	55	187	761	351
K ₃	230	2	122	44	398	0.9	15	143	67	226	682	387
NaK ₁	69	10	206	113	398	1.5	17	119	51	189	718	342
NaK ₂	121	13	173	84	391	0.8	16	124	53	194	723	359
NaK ₃	228	9	100	51	388	1.1	14	137	69	221	638	363
DRFS*												
0.05	14.8	1.8	14.7	6.7	n.s.	0.37	n.s.	13.6	8.5	18.3	40	24.5
0.01	21.1	2.9	20.9	9.5	0.52	19.4	12.0	26.0	57	34.8

* DRFS = Difference required for significance.

lents per 100 grams of dry tissue. In the last two columns of each table totals for cations and anions are expressed as milliequivalents per kilogram of fresh material. With respect to soluble and insoluble fractions the outstanding differences between treatments are common to both bases of expression.

In the mature leaves total soluble cations increased progressively with increasing potassium in both series. On a dry weight basis differences were highly significant except in the comparison between treatments K_1 and K_2 . Differences in individual cation contents were considerable. In each series deficiency of potassium was compensated for in part by increases in calcium and magnesium. Calcium predominated in the K_1 treatment; whereas, magnesium predominated in the NaK_1 treatment. In comparisons between intermediate and high potassium levels, high potassium contents were not nearly balanced by decreased soluble calcium and magnesium contents.

In the expanding leaves total soluble cations were essentially identical in K_1 and K_2 treatments and did not differ significantly between NaK_1 and

TABLE VI

CORRELATION COEFFICIENTS BETWEEN TOTAL INORGANIC CATIONS AND INORGANIC ANIONS IN SOLUBLE AND INSOLUBLE FRACTIONS*

	MATURE LEAVES	EXPANDING LEAVES
Soluble	+ 0.877	+ 0.947
Insoluble	+ 0.819	+ 0.885
Total	+ 0.845	+ 0.750

* Coefficients required for significance for 18 observations—0.05 = .468; 0.01 = .590.

NaK_2 , i.e., in low potassium treatments decreases in potassium were compensated for by increases in calcium and magnesium in the expanding leaves.

Treatment variations in total soluble inorganic anions resulted largely from variations in soluble sulphate and nitrate in both mature and expanding leaves. Soluble sulphate increased in a rather regular fashion with increasing potassium concentrations particularly in the mature leaves. This trend was related in part to an increase in total sulphur content of the leaves and to a greater absorption of the sulphate ion by the whole plant at high potassium levels (5), but at the high potassium levels it was also associated with significant decreases in the insoluble sulphur content. With respect to variations in nitrate content differences in rates of nitrate reduction in the various treatments as well as differences in rates of absorption seem to be involved (5, 6).

One feature shown by the data of tables IV and V is the general inverse relationship between soluble and insoluble components. Also evident is the close correlation between inorganic cations and inorganic anions in soluble and insoluble fractions in both mature and expanding leaves. Coefficients of these correlations are recorded in table VI.

TABLE VII
EXCESS CATIONS, ORGANIC ACIDS AND CARBONATE COMPONENTS

TREATMENT	MILLEQUIVALENTS PER 100 GRAMS OF DRY TISSUE									
	MATURE LEAVES					EXPANDING LEAVES				
	EXCESS CATIONS	ORGANIC ACIDS	HCO ₃ ⁻	CO ₃ ⁼	ORGANIC ACIDS + HCO ₃ ⁻ + CO ₃ ⁼	EXCESS CATIONS	ORGANIC ACIDS	HCO ₃ ⁻	CO ₃ ⁼	ORGANIC ACIDS + HCO ₃ ⁻ + CO ₃ ⁼
	SAP SOLUBLE									
K ₁	137	99	6	105	87	13	77	
K ₂	120	95	12	107	77	21	84	
K ₃	120	97	17	114	97	26	102	
NaK ₁	131	93	7	100	93	14	81	
NaK ₂	133	83	16	99	89	24	83	
NaK ₃	150	94	14	108	107	25	110	
DRFS*	19.5	13.7	4.4	n.s.	14.6	4.3	10.2	
0.05	27.7	19.4	6.3	20.8	6.2	14.6	
0.01										
	INSOLUBLE									
K ₁	164	33	125	158	135	94	110	
K ₂	158	32	127	159	142	100	116	
K ₃	91	26	63	89	75	24	40	
NaK ₁	147	33	106	139	116	75	93	
NaK ₂	131	30	98	128	108	68	84	
NaK ₃	55	24	42	66	60	7	24	
DRFS*	22.9	3.5	13.0	22.3	16.2	11.7	13.0	
0.05	32.5	5.0	18.5	31.7	23.1	16.6	18.5	
0.01										
	TOTAL									
K ₁	301	132	6	125	263	222	13	94	187	
K ₂	278	127	12	127	266	219	21	100	200	
K ₃	211	123	17	63	203	172	26	24	142	
NaK ₁	278	126	7	106	239	209	14	75	174	
NaK ₂	264	113	16	98	227	197	24	68	167	
NaK ₃	205	118	14	42	174	167	25	7	134	
DRFS*	22.7	n.s.	4.3	13.0	19.8	18.2	4.3	11.7	15.2	
0.05	32.3	6.3	18.5	28.1	25.8	6.2	16.6	21.6	
0.01										

* Difference required for significance.

In table VII are shown values for the excess of cations over inorganic anions, total non-volatile organic acids and carbonate in mature and expanding leaves. In each case the total excess of cations was lower in high potassium treatments (K_3 and NaK_3) than in other treatments. It may be seen that these variations were brought about entirely by corresponding variations in the insoluble fraction, which consisted largely of calcium and carbonate. In the soluble fraction from the mature leaves no consistent variations in excess cations were apparent. In NaK_3 , the excess of cations was significantly higher than in K_2 or K_3 , but these differences were not associated with any corresponding differences in organic acids. The soluble fraction from the expanding leaves showed high values for excess cations in the high potassium treatments. These high values were associated with high organic acid contents. Correlation coefficients for the relationship between excess cations and several anion fractions are shown in table VIII. The outstanding relationship was that between carbonate

TABLE VIII

CORRELATION COEFFICIENTS OF RELATIONSHIPS BETWEEN EXCESS CATIONS AND ORGANIC ANION FRACTIONS*

	MATURE LEAVES	EXPANDING LEAVES
Soluble cation excess and soluble organic acids	- 0.116	+ 0.769
Insoluble cation excess and carbonate plus insoluble organic acids	+ 0.955	+ 0.946
Total cation excess and organic acids plus carbonate	+ 0.846	+ 0.750
Insoluble calcium and carbonate	+ 0.984	+ 0.979

* Coefficients required for significance for eighteen observations: $0.05 = 0.468$; $0.01 = 0.590$.

and the excess cations of the insoluble fractions (largely calcium). In the expanding leaves a lower, but highly significant correlation was shown between soluble organic acids and soluble excess cations. In the mature leaves there was no such correlation.

INDIVIDUAL ORGANIC ACIDS

In table IX individual and total sap soluble organic acids are reported as milliequivalents per 100 grams of dry leaf tissue and as milliequivalents per liter of sap. In the mature leaves most of the treatment variations in the sap concentration of total organic acids were associated with variations in total dry weight of the leaves. The result is, higher sap concentrations in low potassium leaves were not significant on the dry weight basis. However, some of the variations in malic and citric acids were considerable, and most of these variations are evident from either basis of expression.

MALIC ACID.—In the mature leaves the principal variations in sap concentration of total organic acids were associated with variations in malic

acid. At low and intermediate potassium levels the concentration of malic acid was lower in series 2 than in series 1. The concentration of this acid was closely related to the concentration of soluble calcium as shown in figure 1. The correlation coefficient is +0.877. The treatments may be divided rather sharply into two groups according to malic acid contents; i.e., treatments K_1 , K_2 , and NaK_1 , had relatively high concentrations, whereas K_3 , NaK_2 , and NaK_3 were relatively low in this acid. These two groups of treatments had relatively high and low calcium contents respectively. For any given treatment, malic acid was much lower in expanding leaves than

TABLE IX

SAP SOLUBLE ORGANIC ACIDS

TREATMENT	MILLIEQUIVALENTS PER 100 GRAMS DRY TISSUE				MILLIEQUIVALENTS PER LITER OF SAP				UN-KNOWN ACIDS AS % OF TOTAL
	MALIC	CITRIC	UN-KNOWN ACIDS	TOTAL	MALIC	CITRIC	UN-KNOWN ACIDS	TOTAL	
MATURE LEAVES									
K_1	55	25	18	99	132	59	47	238	19
K_2	50	20	25	95	104	42	51	197	26
K_3	29	30	36	97	56	56	73	185	37
NaK_1	42	21	30	93	94	44	68	206	32
NaK_2	29	21	33	83	61	42	70	173	40
NaK_3	27	28	37	92	51	57	71	179	40
DRFS*									
0.05	7.1	5.9	9.8	13.7	14.3	12.5	17.6	23.4
0.01	10.1	8.4	13.9	19.4	20.4	17.8	25.0	33.3
EXPANDING LEAVES									
K_1	27	15	22	64	59	33	43	135	34
K_2	18	11	34	63	40	25	69	134	54
K_3	18	25	33	76	36	50	58	144	43
NaK_1	18	11	38	67	38	23	75	136	57
NaK_2	13	13	33	59	29	28	66	123	56
NaK_3	17	24	44	85	32	52	69	153	52
DRFS*									
0.05	7.5	3.3	10.7	8.8	14.8	6.3	24.1	14.1
0.01	10.7	4.7	15.2	12.6	21.0	9.0	34.3	20.1

* Difference required for significance.

in mature leaves. In the expanding leaves, malic acid was increased only by the K_1 treatment. Among the expanding leaf samples this treatment also had the highest calcium content.

CITRIC ACID.—No such simple relationship can be shown between any single cation and the concentration of citric acid. It is noteworthy, however, that in both mature and expanding leaves, and in each series, this acid was significantly higher in the high potassium treatment than at the intermediate potassium level. These differences were all statistically significant, but were particularly outstanding in the expanding leaves. However, citric acid also increased in the leaves of potassium deficient plants in series 1 (K_1).

UNKNOWN ACIDS.—In the mature leaves these acids were lower in treatments K_1 and K_2 than in either of the high potassium treatments. In K_1 , the concentration of this group of acids was lower than in any of the treatments of series 2. In the expanding leaves, K_1 had lower concentrations than any other treatment, whereas NaK_3 had a higher content than treatments other than K_2 and NaK_1 . Attempts to detect oxalic acid in guayule leaves were without success.

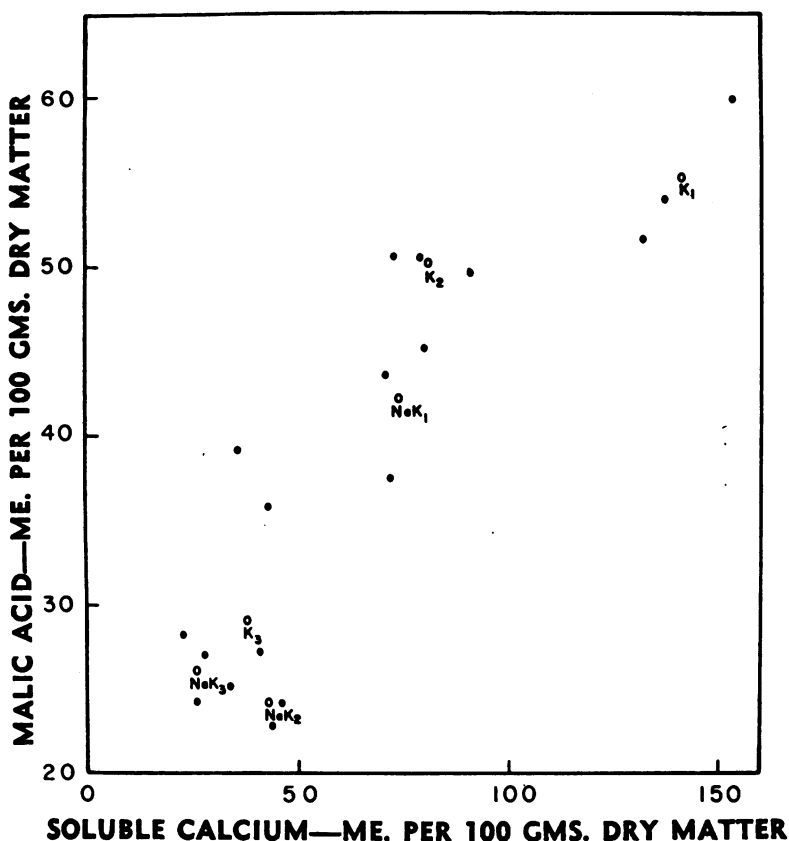


FIG. 1. Relationship between sap soluble calcium and malic acid in mature leaf samples. Small dots represent samples from individual plots. Circles denote treatment means. Coefficient of correlation = +0.877. Required for significance, 0.590 ($n = 18$, $p = 0.01$).

Discussion

In the leaves of guayule the excess of cations over inorganic anions is not nearly balanced by the ether soluble organic acids. In this respect guayule differs from the tobacco plant (22) and from eleven of the twelve species studied by PIERCE and APPLEMAN (21). However, the results for guayule are in agreement with those of the latter workers for cantaloupe. Guayule and cantaloupe differ from the other species studied by Pucher

et al., and Pierce and Appleman in that the leaves contain no oxalate. In guayule, the discrepancy between excess cations and organic acids is accounted for by carbonate. The sum of the total organic acids and carbonate is highly correlated with the excess of cations over inorganic anions, throughout the range of nutrient treatment employed.

The dominant position of insoluble carbonate in the cation-anion balance of guayule leaves is of special interest. There can be little doubt that the carbonate is very largely, if not entirely, in the form of calcium carbonate. In addition to the very high correlation coefficients between insoluble calcium and insoluble carbonate is the fact that calcium was the only cation present in sufficient quantity in the insoluble phase to account for the carbonate. Furthermore, in the mature leaves (table VII) after correcting for the smaller quantities of insoluble magnesium and the insoluble anions other than carbonate, quantities of residual calcium were in very close numerical agreement with quantities of carbonate.

The presence of calcium carbonate deposits in the cells of certain plants has long been known. Meyen, according to DEBARY (7), discovered the cystoliths of the Urticaceae in 1839. A number of species from other families of plants contain less conspicuous deposits. In the Boraginaceae, Cruciferae, Compositae and other families, calcium carbonate has been found in or closely associated with the epidermal hair cells. Yet, in the numerous studies of tissue reactions and buffer systems which have been made, the carbonate system has not frequently been found to be an important component. SMALL (29) emphasized the errors which may result from loss of carbon dioxide from the tissues, particularly when extracted or pressed. He also mentioned calcium carbonate as one of the insoluble calcium salts which may serve a buffering function by precipitation. However, in the tissues from numerous species investigated by Small and his students, pH values of 7 or above were rarely encountered. MARTIN (17) reported the presence of a carbonate system in stems of the bean plant. HAAS (11) obtained alkaline pH values in sap expressed from sweet clover and determined the volume of carbon dioxide which could be liberated from the sap.

In guayule leaves, histological study showed the presence of carbonate in the epidermal trichomes, but no evidence of its presence in other tissues could be found. This conforms to the observations reviewed by DeBary in that calcium carbonate was frequently found in the epidermal hairs or otherwise closely associated with the epidermis. The results of THODAY and EVANS (31) are of interest in this connection. Various solutes were found to be rather highly localized in different tissues or cells. The most striking example was in *Mesembryanthimum* where some cells of the water tissue contained abundant calcium in solution whereas other cells contained soluble oxalate.

In the experiment concerned with the recovery of carbonate from guayule leaf tissues, it was found that rather large variations in pH of the sap expressed from a given leaf sample resulted from variations in the time

allowed for thawing of the frozen material. The pH values increased as the time of thawing was prolonged (table II). Parallel with increasing pH was an increase in carbonate constituents of the sap (largely bicarbonate in this range of reaction). Another result of prolonged standing of the thawed tissues was a decrease in the phosphorus content of the sap. This decrease was accounted for by increased insoluble phosphorus. From these observations it is suggested that the observed bicarbonate and pH values of expressed sap result from a reaction of calcium carbonate with uncombined acids. These reactions take place after a composite mixture of the tissue fluids has resulted from disorganization of the protoplasmic structure by freezing and thawing. It must be considered that in the living state the carbonate is largely localized in the epidermal trichomes and that the reactions of the other cells of the leaf may be considerably more acid than is the reaction of the expressed sap.

It is of interest to apply this interpretation to the analytical results reported in tables IV, V, and VII in an attempt to reconstruct the distribution prevailing in the living leaves. In accordance with this interpretation, the total phosphorus found in the leaves is expressed as H_2PO_4^- . The "bicarbonate" reported in table VII is considered to have arisen from insoluble carbonate. Approximations relative to the forms of other ions are the same as previously. The results of these calculations are shown in table X. It is evident that the relationships shown in table VII are not qualitatively altered and are as significant when expressed in this manner. It may be noted that the numerical agreement between excess cations and organic acids plus carbonate is improved by this mode of expression.

In table X it is noted that the ratio: excess cations/organic acids plus carbonate exceeded unity in all cases except one, indicating a general excess of cations over all anions accounted for. Variations in this ratio indicate this excess was greater in the low potassium samples. However, the pH values were less than 7 for sap expressed from mature and expanding leaves of K_1 and from mature leaves of NaK_1 (table III). Furthermore, the evidence as noted above indicates that these values were somewhat higher than those of the living cells of the mesophyll. At this point it is necessary to recall that no account has been taken of the calcium, magnesium, sulphur, and phosphorus combined in forms other than salts of inorganic acids, non-volatile organic acids and carbonate. It appears that in the low potassium treatments the proportion of cations and anions combined in forms other than those considered was altered. The altered ratio in the low potassium samples could be accounted for by greater amounts of calcium or magnesium, or smaller amounts of sulphur or phosphorus in organic combination. In tobacco leaves (22) it was similarly found that the ratio: excess cations/organic acids exceeded unity, yet the reaction of these tissues indicated the organic acids are not entirely combined. Although there was correlation between excess cations and organic acids in tobacco leaves, the ratio as in guayule leaves was altered with changing mineral contents of the samples.

The proportions of individual acids present in potassium deficient leaves of series 1 were different from those in leaves containing adequate potassium. The expanding leaves of K_1 contained higher concentrations of malic and citric acids and lower concentrations of unknown acids in comparison with expanding leaves of K_2 . Similar variations were indicated in the mature leaves of these plants although these were statistically significant only when expressed in terms of sap concentration. Such variations were not apparent in series 2. These results may suggest that the accu-

TABLE X

CATION-ANION BALANCE OF MATURE AND EXPANDING LEAVES

TREATMENT	TOTAL CATIONS	TOTAL INORGANIC ANIONS	EXCESS CATIONS	ORGANIC ACIDS	CARBONATE	ORGANIC ACIDS + CARBONATE	RATIO: EXCESS CATIONS ORGANIC ACIDS + CARBONATE*
MILLIEQUIVALENTS PER 100 GRAMS DRY MATTER							
MATURE LEAVES							
K_1	493	185	308	132	137	269	1.15
K_2	501	217	284	127	151	278	1.02
K_3	460	244	216	123	97	220	.98
NaK_1	484	198	286	126	120	246	1.16
NaK_2	491	220	271	113	130	243	1.12
NaK_3	456	241	215	118	70	188	1.14
DRFS†							
0.05	31.4	22.0	24.0	n. s.	10.4	17.0
0.01	44.7	31.3	34.1	14.8	24.1
EXPANDING LEAVES							
K_1	413	181	231	80	120	200	1.16
K_2	406	179	227	79	142	221	1.03
K_3	398	218	180	92	76	168	1.07
NaK_1	398	180	218	85	103	188	1.16
NaK_2	391	186	205	75	116	191	1.07
NaK_3	388	214	174	102	57	159	1.09
DRFS†							
0.05	n. s.	16.8	23.3	8.8	13.6	19.2
0.01	23.9	33.2	12.6	19.3	27.4

* Correlation coefficients: Excess cations and carbonate plus organic acids. Mature leaves + 0.839; Expanding leaves + 0.784.

† Difference required for significance.

mulation of malic and citric acids in leaves of K_1 is in some way related to the high soluble calcium contents of these leaves. ILJIN (14), according to PIERCE and APPLEMAN (21), found in general that soluble calcium in plants was highly correlated with malic and citric acids. Pierce and Appleman in comparing different species of plants grown in the same nutrient solution found malic and citric acids to be somewhat correlated with soluble calcium, particularly in the leaves. VICKERY *et al.*, (34) grew tobacco plants in solutions containing various proportions of nitrate and ammonium nitrogen. Leaves of plants supplied with high percentage of nitrate contained high proportions of malic and citric acids, whereas those

of plants supplied with solutions containing mostly ammonium nitrogen had higher proportions of acids of the unknown group. Similar results have been obtained by WADLEIGH and SHIVE (35) for corn and by CLARK (4) for tomato plants. In ammonium culture, the content of total mineral cations including calcium is relatively low. In the present experiment, malic acid of the mature leaves seemed to be related to soluble calcium content throughout the range of treatments. Citric acid in some cases increased in high calcium leaves, but greater and more consistent increases were found in leaves very low in calcium. It should be noted, however, that the plants used in this experiment were relatively young. It is possible that the proportions of individual acids would be altered with age, or even by diurnal variations.

It was not possible to determine whether the hydrogen ion concentration of the leaf cells was altered by potassium deficiency or what effect this treatment had on the proportions of free and combined acids in these cells. However, any alterations in tissue reactions which may have occurred in the potassium deficient leaves could not have resulted from decrease in the excess of total soluble cations, since there was no decrease in these values, nor in the total soluble organic acids. These results differ from some of the results reported by DUNNE (8) for other plants. Plants which do not normally contain large amounts of soluble calcium such as wheat and buckwheat may differ considerably in their response to potassium deficiency, in that sufficient soluble calcium may not be present to maintain sufficient excess of soluble cations. In such cases, a reduction in concentrations of components necessary for the buffer system may be one of the early results of potassium deficiency.

Leaves of high potassium plants invariably had low values for the excess of total cations over inorganic anions. These low values were correlated with low contents of insoluble carbonate, which in turn were directly related to the low calcium contents of these tissues; nevertheless, the total soluble cation and inorganic anion contents of these tissues were invariably higher. In the mature leaves consistent variations in the excess of soluble cations were not found. In the expanding leaves of high potassium plants there were increases in the excess of soluble cations over soluble inorganic anions. These increases were correlated with increases in soluble organic acids. This result is analogous to results obtained by ULRICH (33) in that an increase in soluble cations in excess of soluble inorganic anions was associated with an increase in organic acids. DUNNE (8) found that wheat and buckwheat plants supplied with low calcium solutions had higher values for the excess of soluble cations. The sap from these plants showed increased buffering in the acid range, indicating higher organic acid content. The increase in organic acids was apparently great enough to compensate for the higher cation contents in the sap, since the pH of the sap was the same for plants of high and low calcium contents. This is of interest in connection with the results from guayule, since in guayule leaves it was

not possible to determine whether the reactions of the fluids within the tissue were actually altered.

Summary

1. Cation-anion relationships were studied in expanding and mature leaves of guayule plants which had been supplied with low, intermediate, and high levels of potassium. It was found that the excess of cations over inorganic anions in these leaves was greatly in excess of the non-volatile organic acids present.

2. Highly significant correlations were found for a relationship between the non-volatile organic acids plus carbonate and the excess of cations over inorganic anions. However, this relationship was dependent largely upon variations in insoluble calcium and carbonate.

3. A large proportion of the calcium present in the leaves was insoluble. The insoluble calcium content was closely related to the insoluble carbonate content. The carbonate was highly localized in the epidermal hairs. No oxalate could be found in the leaves.

4. The recovery of carbonate and the distribution of components of the carbonate system between soluble and insoluble phases were compared after subjecting samples to several treatments.

5. Total organic acids and values for excess cations in the leaves were at least as high in low potassium as in intermediate potassium treatments of the same series. There was a greater excess of cations over all anions accounted for in low potassium than in other treatments.

6. In the expanding leaves there was a highly significant correlation between sap soluble organic acids and the excess of sap soluble cations throughout the range of treatments. The principal treatment variations were higher contents of organic acids and excess cations in leaves from high potassium treatments. The higher total organic acid contents of high potassium leaves were largely accounted for by higher contents of citric acid, although in one case the acids of the unknown group also appeared to be involved. In the mature leaves consistent variations in sap soluble organic acids or in excess of soluble cations were not found.

7. In the mature leaves, sap soluble calcium was highly correlated with sap soluble malic acid. Malic acid was generally much lower in expanding leaves than in mature leaves of the same plants, but in expanding leaves too, the highest malic acid content was found in leaves containing the most calcium.

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