CCXXVI. SEASONAL CHANGES IN THE ORGANIC ACIDS OF RHUBARB (*RHEUM HYBRIDUM*)

BY ALLAN ALLSOPP

From the Department of Botany, The Victoria University of Manchester

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THE widespread uses of rhubarb have led to its repeated investigation. Most published works are concerned only with the chemical composition of the plant in relation to its practical value, but as a familiar example of a highly acid plant, rhubarb has also attracted the attention of workers on plant metabolism.

There are numerous accounts of the nature of the acids in this plant. The presence of oxalic acid has been observed by many investigators. Several workers have stated that malic acid forms the greater proportion of the total acid, but only Castoro [1902] presents any real evidence for such a statement. Ruhland & Wetzel [1927; 1929] claimed that the leaves contain oxalic acid, small quantities of *dl*-malic acid and large amounts of *l*-malic acid, while the rhizome contains oxalic and *dl*-malic acids only. They gave no description, however, of their methods of identification or determination of the acids. Citric acid has been obtained from the leaves by Kreis [1916], Besson [1918] and Angerhausen [1920] and also from the rhizome by Bennet-Clark & Woodruff [1935].

Views on the role of the acids in metabolism are as diverse as the accounts of their nature. Steinmann [1917] concluded that the acids originate in carbohydrate metabolism, possibly even as a direct result of photosynthesis. Ruhland & Wetzel [1927; 1929] believe that the organic acids together with ammonia arise in the developing petioles by the deamination of amino-acids passing out from the sprouting rhizome. This deamination, it is held, gives rise to *l*-malic acid, while the inactive acid of the leaves is translocated from the rhizome. Bennet-Clark & Woodruff [1935] found no gain in the content of acid in the whole plant on sprouting. They considered that Ruhland & Wetzel confused an increase in concentration in the young leaves with an increase in the total quantity of acid present in the plant. It was also shown that there is a considerable increase in *l*-malic acid during summer, pointing to its association with carbohydrate rather than with protein metabolism. The small ammonia/malic acid ratio gave no support to the view that malic acid is a product of deamination.

Bennet-Clark & Woodruff carried out determinations of total lead-precipitable acid and *l*-malic acid only, and moreover used too few plants to permit of any statistical treatment of the results. These objections are removed in the present work in which large numbers of plants were used and malic, citric, and oxalic acids were all determined separately.

When the present investigation was virtually completed Pucher *et al.* [1937, 1, 2] published two papers also dealing with the organic acids of rhubarb. Their work concerns the nature of the acids rather than the course of the seasonal changes in acidity of the whole plant, but there is close agreement with the present work whenever the two investigations coincide.

EXPERIMENTAL

Material

The variety used was Linnaeus. On 11 March 1936, six crowns were each divided into about a dozen pieces of approximately the same size. The pieces were washed, freed from dead leaf bases etc., dried and weighed. One piece from each plant was chosen at random and kept for analysis. The remaining pieces were planted in six rows, one row to each original plant. On each sampling date one plant was taken from each row, sampling within the row being at random. The plants were divided into rhizome+roots, and shoot, and after cleaning and determination of the fresh weights of both parts, the further analyses were carried out as described below. The extracts were not bulked but were analysed separately so that six parallel series of determinations were obtained for citric and malic acids. Total acid and oxalic acid determinations were carried out in one only of the six series.

Analytical methods

(a) *Extraction of the acid.* The material was minced and extracted twice with boiling water. To facilitate filtration, pectins were precipitated by addition of alcohol to a final concentration of 70 %. Before alcohol was added, the extract was acidified with HNO_3 to avoid the retention of Ca salts of organic acids in the residue.

(b) *Total malic and citric acids.* These acids were precipitated as Ba salts by the technique of Auerbach & Kruger [1923]. The dry precipitate was transferred to a beaker, crushed with a glass rod and mixed with sufficient dil. H_2SO_4 to liberate the free organic acids. The precipitate of BaSO_4 was filtered off and the malic and citric acids in solution were determined by the highly specific methods of Pucher *et al.* [1934, 2].

(c) *Total acidity.* The organic acids were extracted with ether by the method of Pucher *et al.* [1934, 1]. The total amount of acid was then determined by titration between the pH limits 7.8 and 2.6 using the quinhydrone electrode.

(d) *Oxalic acid* was determined by Bau's [1918] method on aliquot parts of the solutions obtained by ether extraction.

RESULTS

The results of the determinations are expressed in two ways. In the first place it was desired to show whether there was any seasonal change in the actual content of acid in the plant. This can only be demonstrated by referring the acid content to that of the piece of rhizome from which the plant developed. We adopt the convention of Bennet-Clark & Woodruff [1935] in recording the acid content as mg.-equiv. per 100 g. fresh weight of the original piece of resting rhizome (subsequently abbreviated to "per 100 g. o.f.w."). The fresh weights are expressed similarly as multiples of that of the original piece of rhizome. The concentrations of the acids are also of importance, and in this case the results are expressed as mg.-equiv. per 100 g. fresh weight.

In making comparisons on successive dates of either acid concentration or content, the assumption is made that the original pieces of rhizome had precisely the same concentration of acid. There is, of course, a certain amount of variation, which introduces a source of error. The inaccuracy is lessened if the results for each date are the mean of a sufficiently large number of samples. In the present

work the values for citric and malic acids and fresh weight on each date are the means of determinations on six plants. These mean values with standard errors attached are recorded in Tables I, II and III. A difference between successive dates is taken to be significant if the chances against it being fortuitous exceed 20 to 1.

Section 1. *Seasonal variation in content of total citric and malic acids*

The quantities of total citric and malic acids are recorded in Table I. The differences between the citric acid contents of rhizome + roots on successive dates are all significant with the exception of those from December to February and February to March. The December–February difference is far removed from the significant level, but the February–March difference is very nearly significant and may be treated as an actual change.

Table I. *Total citrate and total malate contents of rhizome + roots, shoot and whole plant, in mg.-equiv. per 100 g. o.f.w.*

Date	Mean values $\pm$ standard errors					
	Citrate			Malate		
	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant
March 11	4.93 $\pm$ 0.40	0	4.93 $\pm$ 0.40	5.64 $\pm$ 0.52	0	5.64 $\pm$ 0.52
April 2	3.69 $\pm$ 0.27	1.78 $\pm$ 0.49	5.47 $\pm$ 0.58	3.86 $\pm$ 0.31	1.51 $\pm$ 0.44	5.37 $\pm$ 0.51
May 4	1.41 $\pm$ 0.10	2.03 $\pm$ 0.60	3.44 $\pm$ 0.55	2.15 $\pm$ 0.29	3.06 $\pm$ 0.87	5.21 $\pm$ 1.10
June 9	2.57 $\pm$ 0.37	9.19 $\pm$ 2.58	11.76 $\pm$ 2.45	3.71 $\pm$ 0.45	16.75 $\pm$ 5.38	20.46 $\pm$ 6.17
July 26	9.35 $\pm$ 1.79	35.70 $\pm$ 6.03	45.06 $\pm$ 7.48	10.18 $\pm$ 1.64	206.17 $\pm$ 33.56	216.35 $\pm$ 34.49
Sept. 1	28.69 $\pm$ 5.76	34.45 $\pm$ 11.30	63.14 $\pm$ 16.80	27.44 $\pm$ 6.61	218.20 $\pm$ 63.38	254.64 $\pm$ 69.70
Oct. 12	56.60 $\pm$ 11.11	18.84 $\pm$ 4.88	75.44 $\pm$ 15.81	47.20 $\pm$ 9.94	60.27 $\pm$ 19.36	107.48 $\pm$ 27.73
Dec. 21	26.78 $\pm$ 4.56	0	26.78 $\pm$ 4.56	14.65 $\pm$ 1.48	0	14.65 $\pm$ 1.48
Feb. 10	44.49 $\pm$ 13.33	0	44.49 $\pm$ 13.33	31.74 $\pm$ 8.73	0	31.74 $\pm$ 8.73
March 16	17.10 $\pm$ 5.57	5.40 $\pm$ 1.93	22.50 $\pm$ 7.36	11.76 $\pm$ 3.22	10.29 $\pm$ 3.68	22.05 $\pm$ 6.21

It will be noticed (Table I) that sprouting in spring is associated with a continuous fall in citric acid content until May. The content then increases continuously throughout the summer, attaining a maximum in October, by which time there is twelve times more acid in the rhizome than in the piece from which it developed. A striking fall in December is followed by no further significant change till March, when renewed sprouting is again followed by a sharp fall in acid content.

In the shoot, the only significant changes in citric acid content are from May to June and June to July. There is evidently an active production of citric acid during the summer months, for the maximum content of the leaves is eighteen times greater than that before the commencement of active photosynthesis.

When the total amount of citric acid in the whole plant is considered it is noticed that there is no significant increase from March to April, while from April to May there is actually a significant fall in the content of citric acid. The period of sprouting in the following year is also not accompanied by any increase in the amount of citric acid, but the apparent fall does not quite attain the level of significance.

The changes in malic acid content of the rhizome follow the same general trend as those of citric acid (Table I). Sprouting results in a continuous fall until May, followed by a steady increase to a maximum in October. After a fall in December, the acid content rises again in February. This increase is so near the significant level that it will be treated as an actual change in acid content.

Sprouting in the following spring is once more accompanied by a fall in malic acid content.

The malic acid content of the shoot shows no significant increase until June. Following a rapid increase in July the content remains approximately constant till September, but falls again in October. The malic acid content of the leaves in September is seventy-one times greater than in May.

When the total amount of malic acid in the plant is taken into account it is found that in neither the first nor second periods of sprouting is there a gain in this acid.

The outstanding features emerging from this consideration of the malic and citric acid contents are, firstly that during the summer there is a large increase in the amounts of both acids, and secondly that sprouting in spring is not accompanied by any increase in the quantity of either acid. In both cases the loss of acid from the rhizome is at least sufficient to account for the acid appearing in the developing leaves.

Section 2. Seasonal variation in concentrations of total citric and malic acids

Concentrations of citric and malic acids are given in Table II.

Table II. Concentrations of total citrate and malate in rhizome + roots and shoot, in mg.-equiv. per 100 g. fresh weight

Date	Mean values $\pm$ standard errors			
	Citrate		Malate	
	Rhizome + roots	Shoot	Rhizome + roots	Shoot
March 11	4.93 $\pm$ 0.40	0	5.64 $\pm$ 0.52	0
April 2	4.25 $\pm$ 0.36	8.78 $\pm$ 0.43	4.43 $\pm$ 0.34	6.97 $\pm$ 0.51
May 4	1.76 $\pm$ 0.12	7.86 $\pm$ 0.34	2.76 $\pm$ 0.46	11.91 $\pm$ 1.01
June 9	2.57 $\pm$ 0.28	6.37 $\pm$ 0.60	3.73 $\pm$ 0.28	10.19 $\pm$ 0.70
July 26	4.21 $\pm$ 0.16	2.41 $\pm$ 0.11	4.82 $\pm$ 0.36	14.15 $\pm$ 0.82
Sept. 1	4.89 $\pm$ 0.10	2.29 $\pm$ 0.09	4.70 $\pm$ 0.39	14.13 $\pm$ 1.11
Oct. 12	6.88 $\pm$ 0.20	1.96 $\pm$ 0.09	5.68 $\pm$ 0.31	5.85 $\pm$ 0.84
Dec. 21	5.05 $\pm$ 0.26	0	2.88 $\pm$ 0.21	0
Feb. 10	4.71 $\pm$ 0.33	0	3.48 $\pm$ 0.41	0
March 16	2.09 $\pm$ 0.25	6.23 $\pm$ 0.43	1.37 $\pm$ 0.22	11.93 $\pm$ 0.43

In the rhizome + roots, the concentrations of citric and malic acids fall to a minimum in May and rise to a maximum in October. In both cases, there is a fall in December, followed by no further significant change in concentration till the onset of sprouting, which is again coupled with falls in acid concentration.

The citric acid concentration of the leaves falls continuously until July. There is no further significant change till October when a fall in concentration is recorded.

The malic acid concentration rises until May. A slight fall in June is not significant and is succeeded by an increase in July. The concentration then remains approximately constant till September, but falls steeply in October.

It will be noticed that in April and May the concentrations of malic and citric acids in the young leaves are considerably in excess of those in the rhizome. During the same period the gain in concentration of acid in the leaves is greater than the fall in concentration in the rhizome. Such a relationship might suggest a formation of additional amounts of acid either in the rhizome or developing leaves. But in the previous section it has been shown that there is no increase in the total content of the acids. The smaller fall in concentration in the rhizome

finds its explanation in the great bulk of the latter when compared with the small young leaves.

A comparison of the concentration values (Table II) with those of the total content of acid (Table I) reveals that the amount of acid in the plant increases greatly during summer while the concentration shows relatively little change. This is a result of the rapid growth of the plants, since on any given date the acid content is proportional mainly to the fresh weight, the concentration varying only slightly from plant to plant. The values for acid content and concentration should be compared therefore with the fresh weights, which are set out in Table III.

Table III. *Fresh weights of whole plant, shoot and rhizome + roots, as multiples of the original fresh weight, which is taken as unity*

Date	Mean values $\pm$ standard errors		
	Whole plant	Shoot	Rhizome + roots
March 11	1.0 $\pm$ 0	0	1.0 $\pm$ 0
April 2	1.08 $\pm$ 0.018	0.21 $\pm$ 0.51	0.87 $\pm$ 0.043
May 4	1.07 $\pm$ 0.057	0.27 $\pm$ 0.090	0.80 $\pm$ 0.034
June 9	2.52 $\pm$ 0.50	1.54 $\pm$ 0.429	0.98 $\pm$ 0.063
July 26	16.75 $\pm$ 1.65	14.58 $\pm$ 2.06	2.17 $\pm$ 0.36
Sept. 1	20.82 $\pm$ 5.88	14.99 $\pm$ 4.47	5.83 $\pm$ 1.43
Oct. 12	17.18 $\pm$ 3.59	9.19 $\pm$ 2.18	8.09 $\pm$ 1.45
Dec. 21	5.30 $\pm$ 0.84	0	5.30 $\pm$ 0.84
Feb. 10	8.19 $\pm$ 2.45	0	8.19 $\pm$ 2.45
March 16	9.20 $\pm$ 2.39	0.85 $\pm$ 0.294	8.35 $\pm$ 2.01

In addition to the seasonal change in actual quantity, there is also a marked change in the relative proportion of the two acids. In the leaves, a steady fall in the concentration of citric acid is accompanied by a gradual increase in the concentration of malic acid. From July to September the malic acid concentration is approximately constant and this is also true for the citric acid.

In the rhizome in May, the malic acid concentration is in excess of that of citric acid, but there is a continual increase in the citric/malic acid ratio till December, when the citric acid is considerably in excess of the malic acid. In February there is an increase in malic acid concentration but no significant change in the concentration of citric acid.

Section 3. *Seasonal variation in concentration and content of citric, malic, oxalic and unknown acids, and total acidity of a single plant*

The recorded values for each date in this section do not represent the means of determinations on six plants as in previous sections, but are the result of determinations on one plant only. The concentrations are given as mg.-equiv. per 100 g. fresh weight in Table IV, and the acid content is expressed as mg.-equiv. per 100 g. O.F.W. in Table V.

The values for malic and citric acid contents and concentrations follow the same general trend as those for the six plants, and accordingly require no further discussion.

The oxalic acid content of the rhizome shows a loss in April in connection with sprouting, after which the quantity rapidly increases till in September it is 8.6 times greater than that of the original piece of rhizome. The content falls steeply in October and a slight loss in December is followed by a small increase in February. A small loss in March is accompanied by the renewal of sprouting. The concentration values reveal a sharp fall in April, an increase till August,

Table IV. Concentrations of total citrate, malate, oxalate, total acid and unknown acids in rhizome + roots, and shoot of series C, in mg.-equiv. per 100 g. fresh weight

Date	Citrate			Malate			Oxalate			Unknown acids			Total acid		
	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant
March 11	5.87	0	5.87	7.29	0	7.29	11.60	0	11.60	17.84	0	17.84	42.6	0	42.6
April 2	3.06	7.89	3.94	3.85	8.19	4.67	2.86	7.28	3.68	24.93	13.94	36.06	34.7	37.3	23.77
May 4	1.62	6.88	5.61	4.11	9.50	8.98	9.95	4.20	2.78	24.92	12.52	49.1	40.6	33.1	8.38
June 9	3.21	7.03	13.92	4.48	7.91	16.60	10.00	4.86	9.44	22.71	27.80	113.6	40.4	47.6	65.54
July 26	4.47	2.79	67.85	4.59	13.90	278.9	12.00	8.02	17.54	19.94	6.99	152.0	41.0	31.7	200.75
Sept. 1	4.74	2.14	122.7	3.85	13.60	484.3	8.85	6.64	193.5	18.46	13.22	1120.0	35.9	35.6	603.0
Oct. 12	7.20	1.67	82.5	6.96	5.28	159.5	5.44	9.30	315.0	7.0	20.45	852.0	26.6	36.7	298.6
Dec. 21	4.79	0	15.8	3.16	0	49.5	10.20	0	51.0	11.75	0	150.0	29.9	0	120.7
Feb. 10	5.15	0	47.3	5.37	0	49.5	5.71	0	52.5	13.17	0	270.0	29.4	0	120.7
March 16	1.44	5.15	25.0	1.16	11.90	33.1	3.94	5.44	53.99	3.11	6.71	158.7	9.65	11.1	46.6

Table V. Contents of total citrate, malate, oxalate, unknown acids and total acid in rhizome + roots, shoot and whole plant of series C, in mg.-equiv. per 100 g. O.F.IV.

Date	Citrate			Malate			Oxalate			Total acid			Unknown acids		
	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant	Rhizome + roots	Shoot	Whole plant
March 11	5.87	0	5.87	7.29	0	7.29	11.6	0	11.6	42.6	0	42.6	17.84	0	17.84
April 2	2.62	1.32	3.94	3.30	1.37	4.67	2.46	1.22	3.68	29.8	6.26	36.06	21.42	2.35	23.77
May 4	1.09	4.52	5.61	2.76	6.22	8.98	6.66	2.78	9.44	27.2	21.9	49.1	16.69	8.38	25.07
June 9	3.22	10.7	13.92	4.50	12.1	16.60	10.1	7.44	17.54	40.8	72.8	113.6	22.98	42.56	65.54
July 26	15.15	52.7	67.85	15.9	263.0	278.9	41.5	152.0	193.5	142.0	599.0	741.0	69.45	131.3	603.0
Sept. 1	53.40	69.3	122.7	43.3	441.0	484.3	100.0	215.0	315.0	405.0	1120.0	1525.0	208.3	394.7	200.75
Oct. 12	85.3	24.4	109.7	82.5	77.0	159.5	64.6	136.0	200.6	316.0	536.0	852.0	83.6	298.6	382.2
Dec. 21	24.0	0	24.0	15.8	0	15.8	51.0	0	51.0	150.0	0	150.0	59.2	0	59.2
Feb. 10	47.3	0	47.3	49.5	0	49.5	52.5	0	52.5	270.0	0	270.0	120.7	0	120.7
March 16	16.4	8.60	25.0	13.2	19.9	33.1	44.9	9.09	53.99	110.0	48.7	158.7	35.5	11.1	46.6

a continuous drop till October, a rise in December and a further fall till March. Comparison of the oxalic acid concentrations in the rhizome with those of malic and citric acids shows that the fall in oxalic acid from July to October is associated with increases in citric and malic acids; the decreases in malic and citric acid concentrations in December are accompanied by a rise in that of oxalic acid, while the fall in oxalic acid in February is associated with increases in citric and malic acids.

The content of oxalic acid in the leaves increases gradually through the season and falls in October. The concentration is high in the young leaves, falls in May and apart from a slight drop in September shows a continuous increase till the end of the season. The gain in oxalic acid from September to October is probably connected with the great loss of malic acid at this time, but will not account for all the malic acid disappearing. The increase in concentration of oxalic acid in the young leaves is greater than the fall in the rhizome, but when the total amount of acid in the whole plant is considered it is found that in the first period of sprouting there is no increase in the quantity of oxalic acid, and in the second period a small increase is more than accounted for by a loss of citric and malic acids.

The total acid content of the rhizome falls till May and rises to a maximum in September. A loss of acid in October and December is followed by an increase in February with a further drop in March. The concentration shows a rise from April to July, a fall till October and an increase in December, followed by a slight fall in February and a further fall in March. The content of total acid in the leaves rises continuously till September and falls steeply in October. The concentration is approximately constant throughout the season. In the developing leaves in April the concentration of total acid is much in excess of the fall in concentration in the rhizome. The same is true for the second period of sprouting in March of the following year. In both cases however it will be noticed that there has been no increase in the content of acid in the whole plant.

With the exception of a small increase in April, the unknown acid content of the rhizome is parallel with that of the total acid. This increase in the amount of unknown acid in April is accompanied by a fall in the oxalic acid content. With the same exception, the concentration of the unknown acid is also parallel with that of the oxalic acid. The concentration and content of the unknown acid in the leaves are at first in excess of those of the malic acid, fall below in July and September but again predominate in October.

It is of interest to compare the results of our analyses with those of Pucher *et al.* [1937, 1, 2]. In the leaves of the variety used by us, there are on the whole more citric and unknown acids and less malic and oxalic acids than in the variety employed by the American workers. Although the proportions of the acids are different in the two varieties the seasonal changes in concentration of the individual acids are closely parallel. In both cases the citric acid concentration falls sharply during the summer, while malic and oxalic acids show continual increases. The similarity of the results indicates that the course of metabolism is essentially the same in the leaves of both varieties. Unfortunately no comparison can be made of the seasonal change in acidity in the rhizomes since the American workers publish only one rhizome analysis, dated 26 September. From our analyses for 1 September and 12 October, it appears that there are higher proportions of citric and malic acids and less oxalic acid in the rhizomes of our variety than in that of the variety used by Pucher *et al.*

DISCUSSION

One of the main points emerging from the present research is that during the phase of sprouting, from March to May, there is almost no change in the total acid content of the plant. There is no significant fall in the total quantity of malic acid, but that of citric acid shows a definite decrease. The oxalic acid content also falls, while the unknown acids apparently increase slightly in amount.

Although there is no marked change in total content, a striking redistribution of the acids occurs during sprouting. The total and individual acids decrease in amount in the rhizome but increase in the leaves. This result is most conveniently explained by translocation of acid to the leaves from the rhizome.

During summer, there is a great increase in the quantity of all the acids in the leaves. The acid content of the rhizome also increases continuously during the same period. These results are opposed to those of Ruhland & Wetzel [1927; 1929] who hold that malic acid is produced by the deamination of amino-acids in the petioles of the developing leaves. They also state that there is no further formation of this acid during summer and that translocation is restricted to the time of yellowing in autumn. Our results indicate, however, that there is no new formation of malic acid in the young leaves. The rapid production of the acids during the season of active photosynthesis suggests a connection with carbohydrate metabolism, but there is no evidence as to whether they arise in synthetic or degradative processes. Translocation of acid to the rhizome is evidently continuous throughout the season.

The changes in the relative proportions of the acids can be explained in several ways. They may represent successive stages in a chain of reactions or alternatively may arise independently from the same or different substances. In the latter case the great difference between the malic and citric acid concentrations of the leaf would find its explanation in a variation in either the rates of formation or utilization of the acids. A more rapid translocation from leaf to rhizome of citric acid than of malic acid would also account for the relatively low citric acid content of the leaf and high content of the rhizome. A similar result would be attained if both acids are translocated at rates dependent on diffusion gradients, the relative concentrations in leaf and rhizome depending on the equilibrium $\text{citric acid} \rightleftharpoons \text{malic acid}$ the position of which is the innate peculiarity of the enzyme system of the organ concerned.

The seasonal variation in the acid content of the leaves also finds a ready explanation in the following scheme: carbohydrate $\rightarrow$ citric acid $\rightarrow$ malic acid $\rightarrow$ oxalic acid. Such a series of changes accounts for the fact that the great fall in malic acid concentration in autumn is accompanied by an increase in that of oxalic acid, while that of citric acid undergoes little change. This fall in malic acid content from September to October is of considerable interest in that it is in excess of any gain in the acids of either leaf or rhizome. It must therefore undergo conversion into some other product, possibly carbohydrate, or is lost from the plant as CO_2 .

There is no certainty whether all or one only of the acids are translocated from the leaves to the rhizome. If malic acid is the main acid transported it must undergo rapid conversion into citric acid. The constantly increasing proportion of the latter acid is suggestive of such a conversion.

Both acids undergo a marked fall in concentration in the rhizome from October to December. Here again there must be a conversion into other

substances. Sprouting in spring is accompanied by a production of malic acid from an unknown source, for this acid increases in amount without any corresponding fall in that of any other acid.

These changes in acidity in the rhizome suggest that the acids are in equilibrium with unknown substances, possibly carbohydrates. If the latter were the case, the high concentration of acid in the young leaves would be understandable, the acids providing a convenient starting point for the synthesis of carbohydrates. We have no exact knowledge of the precise changes involved, but the rise and fall in acidity show quite definitely that the acids are playing some active role in metabolism, being converted into products as yet unknown. They are certainly not merely passive end-products allowed to accumulate as the salts of poisonous or unwanted bases as was at one time thought.

SUMMARY

1. Rhubarb leaves and rhizomes contain malic, citric and oxalic acids and acids of an unknown nature, which all show marked changes in concentration and content at different periods of the year.

2. Sprouting of the rhizomes is accompanied by translocation of the acid into the young leaves, with the result that the concentrations of malic, citric, oxalic and total acids rise to higher levels than those of the rhizome. A consideration of the total quantity of acid, however, reveals that there is no increase in the amount of any of these acids on sprouting.

3. All the acids increase continuously in quantity in the leaves during the season of active photosynthesis, suggesting that they arise either as a direct result of this process or indirectly from carbohydrate.

4. There is a steady increase in the quantity of the acids of the rhizome during the summer, which indicates that translocation from the leaves takes place throughout the period of acid formation and is not restricted to the time of yellowing in autumn.

5. A seasonal variation in the relative proportions of the individual acids is also observed. The citric acid concentration in the young leaves is at first higher than that of malic acid, but gradually falls to a low and almost constant level. Meanwhile, the malic acid rises to a maximum concentration which is maintained for several weeks until the onset of yellowing, when the concentration rapidly falls. After an initial fall during the growth of the young leaves, the oxalic acid concentration increases continuously, particularly during the phase of rapid disappearance of malic acid. The unknown acids decrease in concentration when that of malic acid is rising, and show an increase when the concentration of malic acid is falling.

6. In the rhizome, the unknown acids are always in excess of any of the individual acids. Malic and citric acids increase in concentration till the end of summer, reaching a maximum in October. Although the malic acid concentration is at first in excess of that of citric acid, the proportion of the latter increases until finally it is present in greater amount. Both acids suffer falls in concentration in December, but that of malic acid rises again before sprouting. The oxalic acid concentration falls at the end of summer, but increases during the winter, while the unknown acids follow a similar course.

7. These marked changes in acidity, particularly the decrease in concentration of all the rhizome acids during winter, indicate that the acids are playing an active role in metabolism and are not merely passive end-products as is sometimes supposed.

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