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STUDIES IN THE METABOLISM OF CRASSULACEAN PLANTS: CHANGES IN THE COMPOSITION OF *BRYOPHYLLUM CALYCINUM* DURING GROWTH

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(WITH TWENTY-ONE FIGURES)

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Certain peculiarities in the chemical composition of succulent plants, especially those belonging to the family Crassulaceae, have long attracted the attention of plant physiologists and chemists. As early as 1815, HEYNE (**4**) noted that the leaves of *Bryophyllum calycinum* (then known as *Cotyledon calycina*) were intensely sour to the taste in the morning but lost their sour taste toward evening, becoming bitter instead. The outstanding chemical studies of this phenomenon were made 60 years and more ago by ADOLPH MAYER (**7**) who associated the changes in taste with reciprocal diurnal alterations in the concentrations, respectively, of titratable acid and of sugar. He also demonstrated that oxygen is liberated during the deacidification process in light, even when carbon dioxide is excluded, and recognized what he thought to be an isomer of malic acid.

An extensive literature on what is commonly referred to as the crassulacean type of metabolism has since appeared. The papers before 1933 have been carefully reviewed by BENNET-CLARK (**1**) [see also EVANS (**3**), and VICKERY and PUCHER (12)], and there have been a few more recent investigations, notably by WOLF (**16, 17**). The fundamental observations have been repeatedly confirmed, and many additional facts have been recorded; it cannot be claimed, however, that a great deal of light has been shed upon the chemical mechanisms involved, despite the manifest and widely recognized importance of the problem. Nevertheless, the development in recent years of reasonably satisfactory and accurate methods for the analysis of plant tissues and, more especially, the identification of MAYER'S alleged isomer of malic acid (the so-called "crassulacean malic acid") as isocitric acid (**5, 10, 11**) have led to the hope that renewed study might reveal helpful information. In this series of papers, accordingly, the results of analytical investigations, chiefly of the leaves and stems of *Bryophyllum calycinum*,

are to be described; the present paper deals with the changes in composition that occur during normal growth. The experiments were carried out in order to see to what extent the changes in chemical composition of a plant which has a pronounced diurnal variation in organic acidity may differ from those of a plant, such as tobacco, which does not display this phenomenon.

Preparation of samples

Leaves picked from Bryophyllum plants derived originally from a single specimen were placed on moist sand in the greenhouse on September 1, 1940, and, 7 weeks later, the plantlets that had formed on the margins were separated and transplanted. On January 2, 1941, 30 young plants were again transplanted into sand in individual 1-gallon crocks and were thereafter flushed three times a week with a nutrient solution of the composition 0.001 M KH_2PO_4, 0.00225 M $Ca(NO_3)_2$, 0.0041 M $MgSO_4$, 0.0021 M KNO_3, 0.0043 M $Mg(NO_3)_2$ and which contained, in addition, 1 mg. per liter each of boron, manganese, and iron. The solution was adjusted to pH 6.0 by the addition of 0.4 ml. of 1.0 N sulphuric acid per liter. On March 27, 20 plants of uniform height and leaf size were selected and divided into four similar groups of five each. One of these groups was taken for the initial sample of the growth experiment (recorded as zero time).

The samples were harvested at 32, 62, and 95 days, on dates selected according to the weather, a bright cloudless day being chosen. The leaves were broken off at the junction with the petiole, the operation invariably being begun between 12:05 and 12:15 P.M. (standard time) in order to provide as great a degree of similarity as possible with respect to the stage in the deacidification process that had been reached in the different samples. The thick, mature, dark green basal leaves were kept separate from the thinner, smaller and lighter green upper leaves. The separation into groups was arbitrary and largely subjective but permitted comparisons to be made between the composition of mature and of relatively immature leaf tissue when the data are expressed in concentration units. Counts and fresh weights were taken and the tissue was then immediately dried in an oven at 80° C. in rapidly circulating air.

After removing the leaves, the stalk was cut at the sand level and the fresh weight, including the petioles, was determined. The tissue was then cut transversely into thin slices and likewise dried. The total time required until the tissues were placed in the oven was approximately 1 hour.

A similar procedure was followed on each of the sampling dates. However, it was necessary to discard 1 plant each from the 32- and the 62-day samples because it was less well developed than the others and had lost a few leaves; 2 plants of the 95-day sample were discarded for the same reasons. After being dried, all samples of tissue were weighed (crude dry weight) before being broken up, and were then powdered in a Wiley mill and subsequently preserved in closed containers. The fundamental data on the counts and weights are assembled in table I. All analytical determinations were

made upon these samples, the results being obtained as percentages of the crude dry weight and then computed in terms of grams, or milliequivalents in the case of organic acids, per single plant, or in grams per kilogram of original fresh tissue weight (*i.e.*, in concentration units), by means of factors obtained from the fundamental data. The one method of calculation provided a means of comparison between the composition of single plants at different stages of growth, the other permitted comparisons upon a concentration basis as growth progressed.

TABLE I

FUNDAMENTAL DATA ON COUNTS AND WEIGHTS OF SAMPLES OF *Bryophyllum calycinum* PLANTS

Growth period (days)	0	32	62	95
Sampling date (1941)	3/27	4/28	5/28	6/30
Number of plants	5	4	4	3
Number of mature leaves	33	68	124	149
Number of young leaves	69	139	187	140
Total number of leaves	102	207	311	289
Total number of petioles	57	65	85	60
Average length of stem (cm.)	17	39	67	90
Crude dry weight of mature leaves (gm.)	19.2	44.9	75.0	110.9
Crude dry weight of young leaves (gm.)	10.6	39.5	78.9	57.7
Crude dry weight of stems and petioles (gm.)	6.4	24.8	58.9	74.2
Crude dry weight of roots (gm.)	4.9	13.4	23.8	23.8
Fresh weight of mature leaves (gm./plant)	52.9	122.8	208.4	366.0
Fresh weight of young leaves (gm./plant)	25.4	110.2	203.2	181.9
Total fresh weight of leaves (gm./plant)	78.3	233.0	411.6	547.9
Fresh weight of stems and petioles (gm./plant)	14.1	77.1	181.6	267.7

Analytical methods

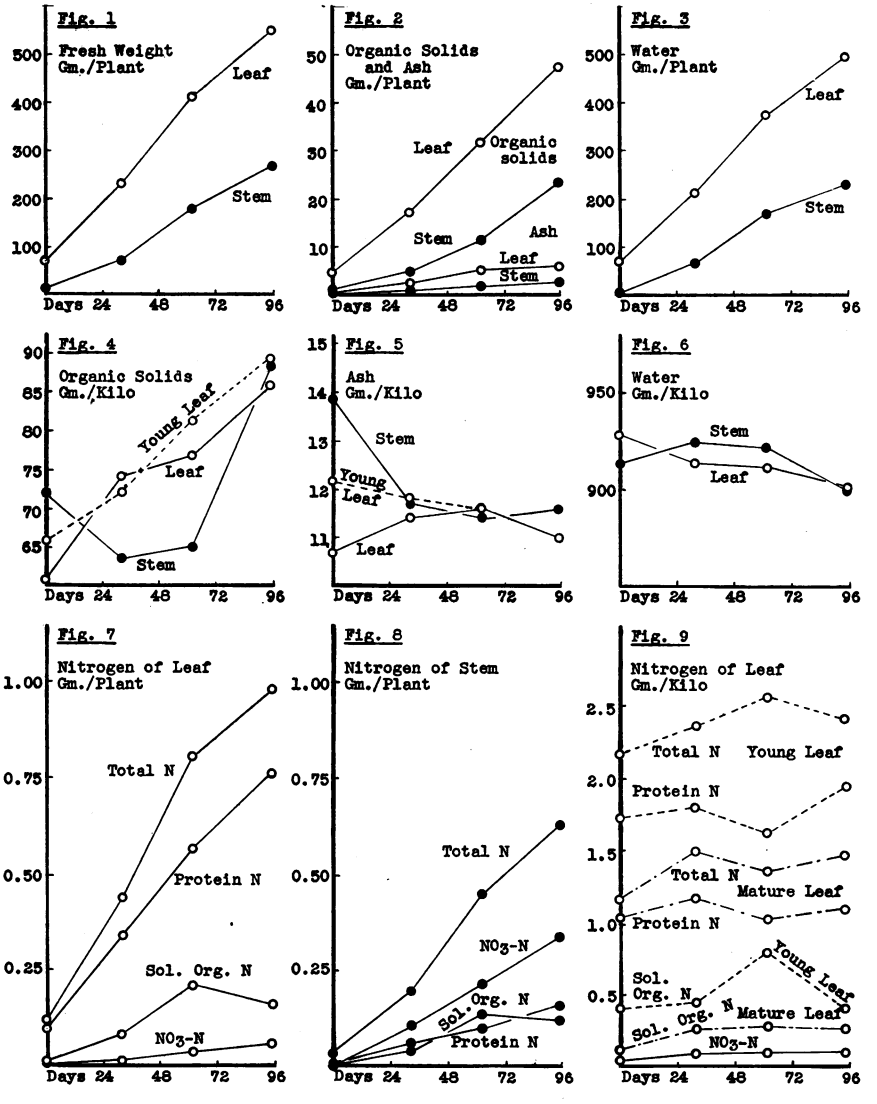
Many of the analytical methods employed in this laboratory have been specially developed and are described in journal papers. A summary of these methods, with full references, is to be found in a recent bulletin from this Station (**15**).

Growth in terms of organic solids and ash

The data for the fresh weight, the organic and inorganic solids, and the water content of both leaf and stem tissue are plotted in terms of grams per single plant in figures 1 to 3. During the period of observation, the growth rate of both the leaves and stems was essentially linear with respect to increase in fresh weight although that of the stems appears to have been slightly accelerated with respect to the rate of accumulation of organic solids as the plants increased in size.

A comparison of the rate of growth of the Bryophyllum plant with that of the tobacco plant is of interest. Data obtained during the season 1933 (**13**) for tobacco plants grown in the field under conditions of commercial production indicated that, 26 days from transplanting, the plants had become well established. At this point they were just passing into a period about 35 days long during which they grew at an essentially linear rate, and

the leaves were found to increase in fresh weight at an average rate of 15 grams per day, the stalks at about 16.6 grams per day. These figures are to be contrasted with an average rate of 5.7 grams per day for the leaves and 2.7 grams per day for the stems of the Bryophyllum plant over the 95-day



period of the present experiment. Clearly the tobacco plant grows much more rapidly than the Bryophyllum plant when compared at presumably analogous points in the course of their respective development.

Figures 4, 5, and 6 show the organic solids, the ash, and the water content of the leaf and stem tissue in concentration units. Although the data for the organic solids of the total leaf tissue indicate a somewhat irregular

increase in concentration, those for the young upper leaves (broken line) follow a nearly linear course. In the stem, on the other hand, the concentration of organic solids decreased at first and then increased rapidly.

The concentration of the ash constituents in the young leaves was higher than the average for the whole mass of leaf tissue in the plants at the start, but the difference became inappreciable as growth progressed. The concentration of ash constituents in the stem decreased rapidly at first, but then levelled off and remained essentially constant after 32 days of growth.

Figure 6 shows that the water content of both leaf and stem tissue remained between the limits 90% and 93% of the fresh weight throughout the experiment. The data for the leaf suggest a small continuous decrease in water content with age whereas the stem increased slightly at first and then decreased. Although this plant is classified as a "succulent," the water content of the leaves does not differ greatly from that of tobacco. Leaves from young growing tobacco plants contain from 89% to 90% of water.

Growth in terms of nitrogenous components

The rate of accumulation of total nitrogen, protein nitrogen, soluble organic nitrogen, and nitrate nitrogen is shown in figure 7. Growth, as measured by total nitrogen, gives a curve that is slightly concave to the time axis although, as measured by organic solids (fig. 2), the curve is linear. It may be inferred that, toward the end of the period of observation, the rate of synthesis of organic substances free from nitrogen gradually increased relatively to the rate of synthesis of nitrogenous organic substances. Nevertheless, the rate of synthesis of protein in the leaf is shown by a curve that departs very little from linearity. The curve for the soluble organic nitrogen (*i.e.*, soluble nitrogen exclusive of nitrate), however, indicates that the rate of accumulation of this group of substances was not maintained in the last interval between observations in spite of the fact that the storage of nitrate nitrogen in the tissue increased regularly. The plants were clearly living under conditions of liberal, if not luxury, supply of nitrate nitrogen, and the alteration in the rate of accumulation of soluble nitrogenous organic substances reflects an alteration in the general course of the metabolism as the plants matured. As is shown in figure 9, the fluctuations in the concentration of the soluble nitrogenous substances in the young upper leaves go far to account for this change; the concentration in the lower older leaves remained essentially constant.

The data in figure 8, showing the rate of accumulation of nitrogen in the stem, are plotted on the same scale as those of figure 7. Although the total nitrogen of the stem increased almost linearly, at each stage of observation slightly more than one-half of the nitrogen was present as nitrate, and only about one-quarter as protein. Both nitrate and protein nitrogen accumulated at a steady rate. However, the soluble organic nitrogenous substances decreased in the stem during the last period of observation as they did in the leaf, again reflecting the change in the general course of the nitrogen metabo-

lism as the plants approached maturity. The large quantity of nitrate suggests a storage function for the stem tissue. Analogous observations have been made for both tomato (**2**) and tobacco (**13**) plants, and the phenomenon is doubtless common.

The concentration of the total nitrogen and of the protein nitrogen in both the young and the mature leaves is plotted in figure 9, together with the concentration of the soluble organic nitrogen. There is a marked contrast between the young and the older leaves; the young leaves were much richer both in nitrogen and in protein and the curves for the latter component, although somewhat irregular, run parallel to each other for most of the period studied. The curve for the concentration of organic soluble nitrogen, like that of the total quantity per plant (fig. 7), reveals a moderate increase followed by a decrease in the last period of observation. The nitrate nitrogen in the whole leaf tissue increased in concentration.

The relative decrease in soluble organic nitrogenous substances coupled with an increase in the protein nitrogen in the plants 95 days old as compared with those 62 days old is of considerable interest. The change is evident in the data for absolute quantities per plant as well as in those expressed on a concentration basis. Examination of the detailed data for the young and for the more mature leaves shows that, during the last interval between observations, the concentration of protein nitrogen in the mature leaves increased very little while that in the young leaves increased materially. However, the concentration of soluble organic nitrogenous substances in the mature leaves remained constant while that in the young leaves decreased. Thus, in the mature leaves, there was a nearly constant relationship between the concentrations of soluble nitrogenous substances and the protein, although both increased in absolute amount per plant. In the young leaves, on the other hand, an increase in the concentration of the protein was associated with a marked decrease in the concentration of the soluble nitrogenous substances.

The careful review by PETRIE (**9**) of the literature of protein synthesis in the plant suggests that protein synthesis is correlated with the level of amino acids in the cell solution but that the relationship is not a simple one; other factors, especially the respiration rate and, accordingly, the rate of liberation of energy, play a part. The present data are not in conflict with Petrie's view of a "drifting steady state" with respect to the relationship between the protein and the amino acids of the plant cells. A reciprocal relationship between the concentration of the soluble nitrogen and of the protein nitrogen is evident in the young leaves although not in the more mature ones. This suggests that the high concentration of soluble nitrogen at 62 days may have been a factor in the relative increase in the concentration of protein nitrogen observed later in the young leaves of the plants.

Figure 10 shows the changes in the concentration of the nitrogenous substances in the stem and should be compared with figure 8 which shows the absolute quantities. There was a sharp increase in the concentration of the

total nitrogen at the start, and much of this arose from the increase in nitrate concentration. Subsequently, the concentration of the nitrate diminished slightly and then remained nearly constant. Nitrate nitrogen made up about one-half of the total nitrogen of the stem throughout the period studied. A high concentration of protein nitrogen is associated with a low concentration of soluble nitrogen in the youngest plants, but this situation is reversed in those 62 days old. In the 95-day-old plants, the concentration of protein nitrogen had increased slightly while that of the soluble nitrogen diminished materially. The reciprocal relationships in the stem are thus analogous to those observed in the young leaves.

Growth in terms of organic acids

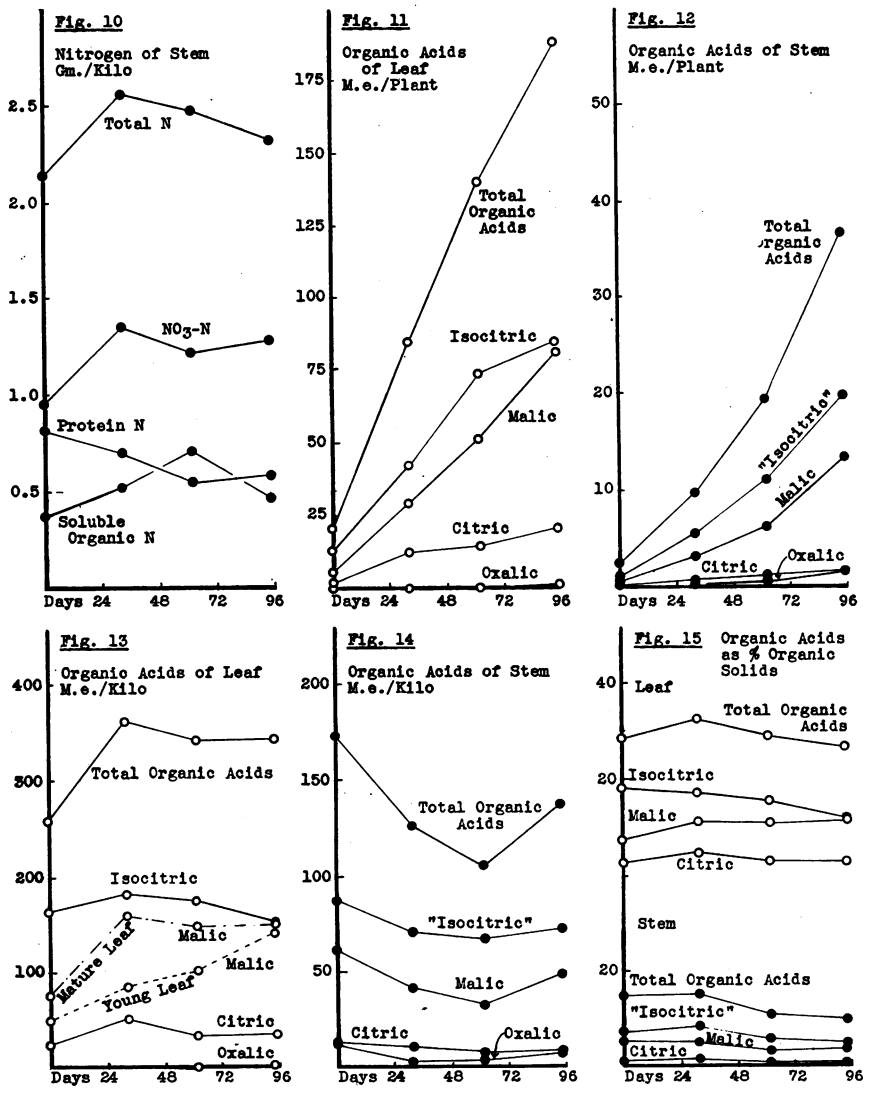
The composition of the leaf tissues with respect to organic acids is shown in figure 11 and that of the stem in figure 12. Bryophyllum leaves are extraordinarily rich in organic acids; the present samples contained from 26% to 32% of the organic solids as organic acids, and these substances are, accordingly, major components from the quantitative point of view. However, the diurnal variation in the organic acids present makes it necessary to specify the time of collection before quantitative statements have precise meaning. During the night, the quantity of acid, especially of malic acid, increases but this process is reversed as soon as the leaves are illuminated so that the level of organic acids has greatly decreased by midday or early afternoon. The present samples were collected at a few minutes past noon on bright sunny days; had they been collected in the early morning, malic acid would doubtless have been the predominating acid component. The smoothness of the curves in figures 11 and 12 suggests that the precautions taken with respect to sampling time were effective.

The total acidity of the leaves, like the organic solids and the protein nitrogen, follows a nearly linear course throughout the experimental period, and this is also true of the malic acid. The rate of accumulation of isocitric acid declined as the plants matured. Citric acid was present in only moderate quantities and the increase during growth was small. Oxalic acid was present in the leaf tissue only in traces, the total amount even in the 95-day plants being so small as to be scarcely apparent on the scale of the diagram.

Organic acids make up a smaller relative proportion of the stem tissue than of the leaf. Figure 12 is plotted on a scale of $3\frac{1}{3}$ times larger than figure 11 in order to show the changes. It will be recalled that the organic solids of the stem amounted to about one-half those of the leaves (fig. 2) but that the stems of even the oldest plants contained less than one-fifth as much organic acid as the leaves. This is the reverse of the situation observed in the rhubarb plant where the petiole was found to be far richer in organic acids (**14**) than the blade.

The general picture of the rate of accumulation of organic acids in the stem is, however, not unlike that in the leaves. The total organic acids increased along a straight line curve for 62 days, but the curve then turned

upwards as the relative quantity of organic solids increased in the more mature stems. The curves for "isocitric" acid and malic acid conform. Neither citric nor oxalic acid was present in more than traces in the stem tissue.



Some discussion is necessary of the meaning of the term "isocitric acid" as used in the analyses of these tissues. It has been shown that by far the greater part of the difference between the total organic acids and the sum of the malic, citric, and oxalic acids in Bryophyllum leaves consists of isocitric acid (**10**). Accordingly, this difference, which in previous papers from this laboratory is denoted as "unknown organic acid," may, in the

particular case of the leaves of Bryophyllum, be used as an approximate measure of the isocitric acid content. The error with this tissue is certainly not important. But we have obtained, as yet, no direct evidence that isocitric acid is present in the stems of this plant. It seems a reasonable assumption, however, that the qualitative composition of the stem will not differ greatly from that of the leaf and, accordingly, the proportion of unknown organic acids in the stem has been provisionally denoted as "isocitric acid." Analytical methods for isocitric acid have been developed during the past two years in other laboratories (**5, 8**). These methods, however, depend on the use of specific enzymes, and their application to the analysis of plant tissues has not yet been made in this laboratory.

Figure 13 shows the organic acids of the leaf tissue expressed in concentration units. During the first 32 days of growth, there was a marked increase in the concentration of the total acids much of which arose from the increase in malic acid; subsequently the concentration diminished moderately. The behavior of malic acid in the mature and in the young leaves was different. The young leaves showed a steady increase in malic acid throughout the experimental period, but the mature leaves increased in malic acid only in the first interval between observations.

The behavior of the concentration of the acids in the stem tissue is shown in figure 14, which is plotted on a scale twice that of figure 13. The total acids decreased for the first 62 days and then increased, and both malic acid and isocitric acid shared in the change. The small concentrations of citric and oxalic acids present altered very little.

Figure 15 shows the organic acids of both leaf and stem tissue as percentages of the organic solids. Isocitric acid is the predominant organic acid and was present to the extent of 18% of the organic solids of the leaves of the youngest plants but the proportion decreased as the plants matured. Young plants are thus obviously to be preferred for the preparation of isocitric acid in quantity. With such material, the relative proportion of isocitric to citric acid is greatest, a circumstance favorable for the separation by chemical means, and greater yields would be anticipated. It would also be more economical of time to work with young plants.

The data for the concentration of the acids in the organic solids of the stem are plotted in the lower part of figure 15. As has already been pointed out, this tissue is relatively low in organic acids and the acidity diminishes with increasing age. The stems of the young plants contained 15% of the organic solids as organic acids and this proportion dropped to 10%. "Isocitric acid" made up about 8% of the solids of the young stems and 5% of the oldest. The proportion of malic acid was somewhat less in each case.

Growth in terms of carbohydrates

Figure 16 shows the rate of increase in total and unfermentable carbohydrates in the leaf tissue, the data representing the reduction measured with the Shaeffer-Somogyi sugar reagent, and being expressed in terms of

glucose. The leaf tissue is moderately rich in sugar, the composition, expressed as percentage of the organic solids, ranging from 8.6% to 6.8% in the mature leaves, and from 3.7% to 4.9% in the young leaves. The present figures represent, of course, only the condition at the time of collection of the samples with respect to a component that fluctuates within fairly broad limits during the day. However, the rate of accumulation of total carbohydrate was essentially linear over the period of observation, suggesting that the samples were taken at comparable stages in this metabolic process.

The component designated unfermentable carbohydrate represents a titration for sugar carried out after treatment of the extract from the leaf with yeast. Clearly, a substantial part of the total carbohydrate consists of substances that are not removed by this treatment, and it is well known that components of similar behavior are commonly found in leaf tissues. The rate at which this type of carbohydrate accumulated seemed to increase moderately as the plants developed.

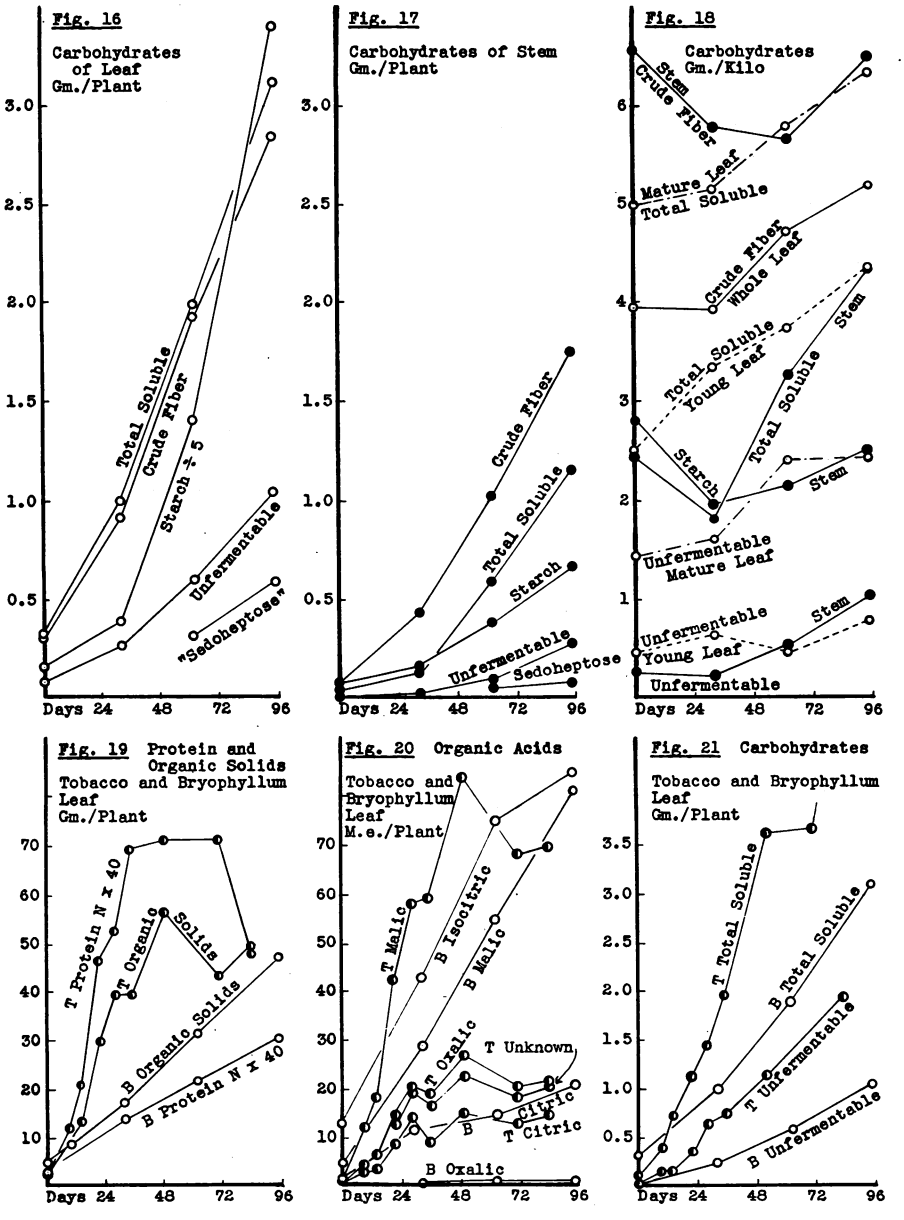
The quantities of crude fiber, determined by the conventional analytical method, are also shown in figure 16. This material doubtless consists mainly of cellulose and the rate of accumulation serves as an approximate measure of the rate of growth of the cell walls. The curve is, within the limits of error, linear throughout the period of study. It is of interest that the quantities of crude fiber in the leaf tissue are nearly identical with the quantities of soluble carbohydrates in each sample. However, no significance should be attached to the coincidence of the curves. No such agreement was observed in the case of tobacco leaves; in these, the slopes of the analogous curves were quite different.

The chief carbohydrate component of the leaf tissue was starch. The analytical values have been divided by 5 in order to bring them within the scale of figure 16, and it is to be noted that the rate of accumulation of starch was continually accelerated as the plants matured. By far the greater part of the starch was found in the older leaves and it is clear that the rate of growth, as measured by the accumulation of starch, is a function quite different from the rates as measured, for example, by the organic solids or the protein nitrogen, both of which were essentially linear. As the plants increased in age, the leaves became increasingly efficient with respect to the synthesis, or at least the storage, of starch.

The data plotted in figure 17 shows that the stem is, in general, lower in carbohydrates than the leaves. The values for total carbohydrate range from 3% to 5% of the organic solids. Unfermentable carbohydrate was present although in relatively small proportion. The rate of accumulation was slow at first but then followed an approximately straight line curve.

The most important carbohydrate component of the stem tissue was cellulose as is shown by the curve for the crude fiber. The rate of deposition of cellulose was slow at first, but proceeded subsequently at a linear and more rapid rate. However, there was less crude fiber in the stem than in the leaves throughout the period of observation. This is in marked contrast to the

behavior of the tobacco plant, the woody stem of which soon exceeds the leaves in quantity of crude fiber present and, at maturity, contains ten or more times as much.



The stems contained only moderate quantities of starch, less indeed than of soluble carbohydrates, and the rate at which starch appeared conforms with the rates for the soluble carbohydrates. There would appear to be little connection, therefore, between starch synthesis in the stem and that in the leaves.

The concentration of the carbohydrate components in terms of fresh weight is shown in figure 18. In the mature leaves, the total carbohydrates increased moderately with age, but, in the young leaves, the rate of increase was somewhat greater. However, the concentration was invariably much higher in the mature than in the young leaves. The unfermentable carbohydrate components were also present in higher concentration in the mature than in the young leaves; there was a moderate increase in the mature leaves with age, but the concentration in the young leaves remained fairly constant throughout.

The concentration of total carbohydrate in the stem dropped sharply during the first 32 days, but subsequently increased to approximately twice the minimal value.

The concentration of crude fiber in the entire leaf tissue is also shown in figure 18; there was no change during the first 32 days of growth but, subsequently, the proportion increased. The behavior of the crude fiber in the stem tissue was quite different; it decreased in relative proportion at first, remained nearly constant for a considerable period and then increased. The concentration changes of fiber in the stem were thus analogous to those of the total organic solids (fig. 4) and the total organic acids (fig. 14).

The concentration of the starch in the leaves has not been plotted in figure 18 since the values are outside the scale of this diagram. In the young leaves it ranged from 8.8 grams per kilo in the plants at zero time to 17.0 grams per kilo in those 95 days later. The corresponding figures for the mature leaves were 11.1 and 24.7 grams per kilo. The values for the stem are shown, however, and indicate that the stems were relatively richer in starch at zero time than they later became. The changes in concentration conform moderately well with those of the total carbohydrate in the same tissue.

Sedoheptose

In figures 16 and 17, an additional component of the soluble carbohydrate fraction is shown at the bottom of the diagram and is marked "sedoheptose." Sedoheptose was discovered by LaForge and Hudson (**6**) in 1917 in the leaves of *Sedum spectabile*. It is characterized by the fact that the reducing power of a solution of the sugar is diminished by approximately 80% of its original value when the solution is heated with 1% hydrochloric acid. The change is the result of the loss of one molecule of water which gives rise to the formation of an anhydride or sedosan; the reaction appears to be an equilibrium. Bennet-Clark (**1**) has employed a method based on this change in reducing power on treatment with acid to detect sedoheptose in the leaves of succulent plants, among them *Bryophyllum calycinum,* and has further noted, in the case of *Sedum praealtum,* that the sugar component which has this property undergoes wide diurnal variations in amount, these variations being reciprocal with those of the acidity. Wolf (**16**), however, was unable to confirm this in the case of *Bryophyllum calycinum;* rather, he demonstrated reciprocal variation of the fermentable carbohydrates and

of the starch as against the changes in the acidity. Nevertheless, he secured evidence of the presence of sedoheptose in Bryophyllum leaves by the isolation of a phenylosazone that corresponded closely in properties to that described by LAFORGE and HUDSON.

A brief study of the reducing power of extracts from Bryophyllum leaves confirmed the presence of a carbohydrate which was diminished in reducing power when the extract was boiled with dilute acid. These analyses were carried out on extracts that had not been treated with yeast; otherwise lower and erratic results were obtained. Experiments in which sulphuric acid of concentrations in the range 0.2 to 1.0 N were used showed that a concentration of 0.35 N was most satisfactory for the formation of the anhydride ring. With this reagent, a maximal diminution of the reducing power was secured after the solution had been boiled for 30 minutes. No further loss occurred after the solution had been boiled for 30 additional minutes. Weaker acid reagents required longer times while a reagent as strong as 1.0 N gave smaller diminutions probably because of the hydrolysis of complex carbohydrates.

The diminution of the reducing power, expressed as glucose, is plotted in figure 16 as "sedoheptose" in spite of the fact that it is known that the anhydride formation with pure sedoheptose is incomplete. LAFORGE and HUDSON state that the equilibrium occurs when 80% of the sugar has been converted, while BENNET-CLARK observed 75%. Furthermore, the relationship between the sugar reagent standardized in terms of glucose and the quantity of sedoheptose reduced is unknown. Because of these two uncertainties, it seemed best to plot the actual quantities of reagent reduced, in terms of glucose, and to postpone the interpretation in terms of the quantities of sedoheptose involved.

With these restrictions of the meaning of the term defined, "sedoheptose" was observed in significant amounts only in the mature leaves of the 62- and 95-day plants. In these, it made up a substantial part of the unfermentable carbohydrate, almost certainly a larger part than is suggested by the relative positions of the curves drawn in the figures. The more mature leaves of the youngest plants gave evidence of only a trace of this component, while the young leaves of all save the 95-day plants gave either an increase in reduction when the extracts were heated with acid or no change. The stems of the 62- and 95-day plants also showed evidence of a trace of "sedoheptose" but, if any were present in the stems of the 0- and 32-day plants, it was not detected since the reduction was increased slightly by the treatment with acid. Clearly, therefore, "sedoheptose" is a component that could be demonstrated only in the mature leaves of fairly old plants and, possibly, in the stems of the same plants.

Composition of Bryophyllum tissues

Table II shows estimates of the quantitative composition of the leaf and stem tissue of these samples of Bryophyllum plants. The data for protein

TABLE II

COMPOSITION OF BRYOPHYLLUM PLANTS

Figures not otherwise designated are grams per single plant.

	Leaf				Stem			
Growth period (days)	0	32	62	95	0	32	62	95
Protein (N × 6.25)	0.619	2.181	3.456	4.756	0.075	0.337	0.625	0.994
Soluble nitrogenous components (N × 10)	0.150	0.780	2.13	1.62	0.050	0.390	1.29	1.22
Starch	0.810	2.07	7.02	12.15	0.040	0.153	0.393	0.668
Crude fiber	0.310	0.913	1.94	2.85	0.093	0.447	1.03	1.75
Ether extract	0.132	0.534	0.992	1.48	0.015	0.068	0.147	0.373
Soluble carbohydrate	0.331	1.003	1.970	3.116	0.035	0.140	0.598	1.165
Isocitric acid	0.828	2.736	4.685	5.398	0.079	0.349	0.707	1.272
Malic acid	0.361	1.980	3.465	5.466	0.057	0.213	0.408	0.886
Citric acid	0.128	0.777	0.930	1.323	0.012	0.055	0.080	0.120
Oxalic acid	0.002	0.006	0.033	0.063	0.007	0.014	0.034	0.082
Total known organic solids (estimated)	3.671	12.98	26.62	38.22	0.463	2.166	5.312	8.530
Total organic solids (determined)	4.80	17.25	31.83	47.44	1.02	4.90	11.82	23.55
Unknown organic solids	1.18	4.27	5.21	9.22	0.557	2.73	6.51	15.02
Unknown organic solids as percentage of total organic solids	24.6	24.7	16.4	19.4	54.6	55.7	55.1	63.8
Inorganic solids	0.84	2.66	4.77	6.01	0.196	0.907	2.075	3.10

are computed from the protein nitrogen by multiplication by the conventional factor 6.25, a procedure that is admissible in the absence of information on the actual nitrogen content of the tissue protein. The estimate of the quantities of soluble nitrogenous components is made by multiplying the data for non-protein soluble nitrogen, exclusive of nitrate, by the factor 10. This is doubtless an overestimate since the average nitrogen content of the substances in this group, presumably amino acids, amides, polypeptides, and basic substances such as choline, purines, and basic amino acids is greater than 10%. However, it has been found, in other samples of Bryophyllum leaves, that from 35% to 40% of the soluble nitrogen is normally present as amino nitrogen; thus a considerable part of the substances concerned probably does consist of amino acids and the factor 10 can be used for these without grave error.

Table II also gives data for the ether extract of the samples. This was obtained by the conventional procedure and represents chlorophyll, lipid pigments, and other fat-solvent soluble material; probably only a small part of it consists of true fat.

The quantities of organic acids were computed from the analyses in terms of milliequivalents by the use of the appropriate factors.

The sums of the estimates are shown, together with the determined quantities of organic solids; the difference between these thus represents that part of the organic material concerning which no qualitative information is available. In the leaf tissue, this unknown part amounts to from one-quarter to one-sixth of the organic solids. In the stem tissue, on the other hand, the unknown part makes up appreciably more than one-half of the total, and suggests that a number of major components await detection and determination. Nothing but speculation can be offered concerning these unknown components in either leaf or stem tissues. However, such substances as glucuronides of the pectin type are probably present in the leaf and complex carbohydrates such as hemicellulose in the stem. Clearly, much remains to be learned regarding the qualitative composition of the tissues of this plant.

Comparison of Bryophyllum with tobacco

In figures 19, 20, and 21 are shown data taken from a study of the rate of growth of the tobacco plant (**13**) plotted together with a few of the more important items of the present data on the Bryophyllum plant in order to afford a comparison of the behavior of the two species. Figure 19 shows the organic solids and the protein nitrogen of the leaf tissues, these being selected as perhaps the most clearly illustrative of the relative rates of growth. The protein nitrogen values have been multiplied by 40 in order to obtain curves that can be plotted on the same diagram with the solids. Zero time for the tobacco plants is arbitrarily taken as the point at which the composition with respect to nitrogenous components was essentially identical with that of the youngest Bryophyllum plants. This occurred 26 days after the tobacco seedlings had been set in the field when the plants had become well established

and were just beginning to grow at a rate which followed a nearly linear course for the next four or five weeks. It is assumed that, during this period, the plants were passing through a phase of growth analogous to that of the present series of Bryophyllum plants. At all events, this was the purely vegetative stage in the growth period of the tobacco plants. The first samples of inflorescence tissue, mainly flower buds, were collected from the tobacco plants at 61 days from transplanting (*i.e.*, at 35 days on the scale of figures 19 to 21) and subsequent samples contained increasing quantities of flowers and developing seed pods. The change in the course of the metabolism, as the plants passed into the reproductive phase, is illustrated on the curve for the leaf protein which flattened out and then fell as protein was withdrawn from the leaves during the ripening of the seeds. There was also a loss of organic solids from the leaves. The main point of interest in the present comparison is, however, the contrast between the relative rates at which leaf protein and leaf organic solids were formed during the first 40 days of growth of the tobacco plant and their behavior in the Bryophyllum plant. Clearly, the tobacco leaf is considerably richer in protein, in relation to the organic solids, than is the Bryophyllum leaf, while the rate of growth, as measured by the rate of accumulation of the leaf protein, is about four times as rapid. The rate of growth, as measured by the organic solids of the leaf, is about twice as great.

Figure 20 shows the composition of the leaves of the two species with respect to organic acids. In the tobacco leaf, malic acid is by far the predominating acid component, and the curve follows in fairly close detail that for the organic solids in figure 19. Oxalic acid is next in relative importance as a component, and the "unknown" organic acids are present in closely similar amounts. The curve for the "unknown" acids of the tobacco leaf should be compared with that for isocitric acid in the Bryophyllum leaf, the main acid component of this species. Both curves depend upon analytical data obtained in the same way, the difference being that the "unknown" acids of the Bryophyllum leaf have been shown to be comprised largely of isocitric acid. Whether or not isocitric acid occurs in tobacco leaf has not been demonstrated. However, observations on the organic acid esters of high boiling point obtained from tobacco leaf tissue suggest that isocitric acid can be present only in traces if at all.

Citric acid is a minor component of both the tobacco leaf and the Bryophyllum leaf, and the curves for the rate of accumulation in the two species are closely similar; in fact this is the only point in which the two are closely alike.

The curve for malic acid in the Bryophyllum leaf represents the composition with respect to this component at noon on sunny days. Had the samples been collected earlier in the day, the curve would, doubtless, have been displaced vertically upwards and might also have had a somewhat different slope. Nevertheless, the rate of accumulation of malic acid in this plant, as a function of age and with this restriction on the time of collection, is clearly less than it is in the tobacco plant.

The curves for the total organic acidity of the two species are not plotted in figure 20; they show, however, that the tobacco plant accumulates organic acids in the leaves, during the approximately 40-day period of its maximal growth rate, somewhat more rapidly than the Bryophyllum plant. However, the check that is placed on the development of the tobacco leaf by the onset of the reproductive phase brings the synthesis of organic acids to a stop, while accumulation of acids in the Bryophyllum leaves continues as is shown in figure 11. Nevertheless, if observations on Bryophyllum leaves were made in the early morning, there is little doubt that the rate of accumulation of total acids would closely approach that of the tobacco leaf.

The qualitative composition and, with the exception of citric acid, the relative rates of synthesis of the organic acids in the two species are entirely unlike. The differences with respect to oxalic acid and isocitric acid are perhaps the most striking. Oxalic acid is an important component of tobacco leaf tissue but is present in Bryophyllum leaves only in traces. The reverse is true for isocitric acid. The metabolic systems that lead to the synthesis of organic acids in the two plants are thus entirely unlike; although there are, doubtless, certain features common to the two, since malic and citric acids are components of both, the details of the chemical mechanisms in which organic acids share are manifestly widely different.

Figure 21 shows the relative rates of accumulation of soluble carbohydrates in tobacco and Bryophyllum leaves. Although the type of tobacco plant described (the so-called Connecticut shade-grown tobacco) is characterized by a somewhat low level of carbohydrate content, particularly of starch, it is obviously a species that synthesizes soluble sugars in the leaves more rapidly and in larger quantities than the Bryophyllum plant. Much of the unfermentable carbohydrate of the mature Bryophyllum leaf is doubtless sedoheptose but this is certainly not true of the tobacco leaf, although unfermentable carbohydrates make up a large part of the soluble sugars in this species. Thus, both qualitatively and quantitatively, the composition of the two kinds of leaves is widely dissimilar.

Summary

The composition of the leaf and stem tissue of *Bryophyllum calycinum* plants, harvested at noon on sunny days at intervals over a period of 95 days, has been determined in order to obtain fundamental data upon the rate of growth of a plant which is characterized by a pronounced diurnal variation in organic acid content. The composition is recorded in terms of grams per plant. The rate of accumulation of the fresh weight and of many of the components, in particular the organic solids, the ash, the water, the protein, the nitrate nitrogen, the soluble carbohydrates, the crude fiber (cellulose), the total organic acids, and the malic acid followed essentially straight line curves throughout the period of observation. On the other hand, the total nitrogen followed a curve somewhat concave to the time axis, as was true also of the isocitric acid and citric acid, suggesting a gradual slowing of the rate

of accumulation of these components, while the starch of the leaves, by far the most plentiful known component of this tissue, followed a curve markedly convex to the time axis indicating an increase in relative capacity for the storage of starch as the leaves matured.

The data have also been computed in concentration units, namely in grams per kilo of fresh weight of the tissues. These curves show a moderate degree of irregularity in the relative concentrations of the various components at different stages of growth.

Comparison of the data with similar results for the tobacco plant, at an analogous period in the life cycle, showed marked differences in the rate of accumulation of most of the components. The tobacco plant grows much faster as measured by almost all of the criteria. The most important exception is in the rate of accumulation of organic acidity; isocitric acid is formed almost as rapidly by the Bryophyllum plant as malic acid is by the tobacco plant, and citric acid is formed at almost equal rates in the two species. On the other hand, oxalic acid, which is a major organic acid component of the tobacco plant, is present only in traces in Bryophyllum.

Isocitric acid is the predominating organic acid component of Bryophyllum leaf tissue and is present in young leaves to the extent of about 18% of the organic solids; such tissue is accordingly valuable for the preparation of this rare acid in quantity.

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