

RELATION OF AGE AND OF SEASONAL CONDITIONS TO
COMPOSITION OF ROOT, PETIOLE, AND LEAF
BLADE IN RHUBARB

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(WITH FIVE FIGURES)

Introduction

Members of the genus *Rheum* have been employed in medicine from a period antedating authentic history, and the general distribution of most of the species throughout Europe and North America is due primarily to such use. The employment of rhubarb as an article of food is relatively recent, the earliest reference to such use found by STURTEVANT (HEDRICK 17) bearing the date 1778; but its use appears to have become rather general shortly after 1800. Excellent descriptions of the several species are given by various writers (3, 7, 19) in connection with directions for the cultural treatment of the crop, and there is a rather extensive literature dealing with the chemical composition of the root and the physiological action of the various constituents isolated therefrom which has recently been reviewed in connection with a comprehensive reexamination of the root (35). The older literature dealing with the use of the plant in medicine has been exhaustively reviewed by HEUBERGER (18). Such chemical studies as have been made of the aerial parts have been concerned with the determination of the food value of the petioles or with the possible toxicity of the leaf blades when employed as food. Apparently no comprehensive physiological or biochemical study of the seasonal development of the entire plant has hitherto been made.

In many respects the rhubarb plant (*Rheum hybridum* was the species studied) lends itself exceptionally well to such investigation. The large size of the several parts and the extremely rapid development of the enormous leaf blades and petioles make it suitable for studies for which large quantities of material and frequent collections are necessary. The size of the leaves facilitates marking, so that collection of material of known age is readily possible. The high acidity and low carbohydrate content of the aerial parts are exceptional, and suggest that the metabolic processes of the plant possess features of interest. In connection with studies upon the food value and cooking and canning qualities of rhubarb, some of which have been reported elsewhere (14), it was found that the physiology of the plant presented some unique features. It was therefore considered advisable to undertake biochemical studies for the light which the results might throw upon the problems of its utilization in cooking and in canning, as

well as for their general physiological interest. The present paper presents the results of this study.

Review of literature

The literature dealing with the chemistry of rhubarb is concerned, as already indicated, with the food value of the aerial parts or the therapeutic effects of the constituents of the root, and not with the biochemistry of growth and development of the entire plant or with the effect of climatic conditions or plane of nutrition in modifying the character or degree of these changes. The work of STEINMANN (32) was devoted to the determination of the titratable acidity of the petiole and leaf blade at various stages of development, to the effect of illumination and darkness upon the acid content, and to the gradient of titratable acidity between leaf mesophyll, veins, and petiole. He reached the conclusion that oxalic acid may be a product of photosynthesis, playing a rôle analogous to that of carbohydrates in ordinary green plants. RUHLAND and WETZEL (27) have reported studies upon the plant which they consider indicate that the high acid content is the result of deamination of proteins rather than of partial oxidation of carbohydrates.

There is an enormous literature dealing with the annual cycle of chemical exchanges between leaves and storage organs of woody perennials, and particularly of fruit trees, from which the general character and sequence of these changes can be rather completely determined. This literature has been excellently summarized by GARDNER, BRADFORD, and HOOKER (16), and by CHANDLER (13). The degree to which the results of such studies apply without modification to the rhubarb plant is questionable, since production of flower stalks and fruits is normally prevented in cultivation, storage of reserves is confined to the underground rhizome and roots, and the aerial parts are remarkable for their consistently high acidity and low content of sugars and acid-hydrolyzable polysaccharides at all stages of their development. In consequence the physiology of the plant would be expected to present some points of contrast with that of woody stemmed perennials and trees on the one hand, and with that of annuals on the other.

There is a considerable literature dealing with the chemistry of development of foliage leaves, chiefly of herbaceous perennials and trees. The results reported by OTTO and KOOPER (24, 25), TUCKER and TOLLENS (34), SWART (33), and LECLERC DU SABLON (15) are typical. SWART found that the fall of the leaf in autumn was preceded by a decrease in protein and in nitrogen, phosphorus, and potassium. He reviewed the older literature in considerable detail and presented analyses upon leaves of a great number of trees and perennial shrubs made at two stages, namely, when the leaves

were normally green and when autumnal coloration had set in. In all cases the development of coloration is accompanied by very marked decrease in total nitrogen, potassium, and phosphoric acid.

TUCKER and TOLLENS (34) collected leaves of *Platanus occidentalis* at intervals of 3 to 4 weeks from June 13 until November 5, and determined water and ash content and the composition of the ash. The water content was highest in the first sample and decreased gradually up to October 8, then rose slightly in the final sample. Ash increased throughout the series. The percentage of silica, calcium, magnesium, and chlorine present in the ash increased steadily through the series; phosphoric acid and potash were highest in the initial sample and fell off gradually up to September 7, then more rapidly to values less than one-half those of the initial sample. Nitrogen showed a similar but more pronounced decrease to one-fourth its original amount. TUCKER and TOLLENS reduced their results to terms of equivalent leaf area, so that the decreases are absolute and not relative. As WEHMER (38) had suggested the possibility that constituents of the ash were removed from the older leaves by rains, a portion of the tree was protected by a water-tight cover during rainfall from October 8 onward. The differences between protected and unprotected leaves were without significance. The reduction in potassium, phosphoric acid, and nitrogen was therefore due to transport from the leaves into the wood, not to leaching by rains.

LECLERC DU SABLON (15) made determinations of sugars and total carbohydrates, total nitrogen, fat, and water in root, stem, and leaf of chestnut, pear, quince, peach, and willow trees and in raspberries at intervals of 30 to 35 days throughout the year, finding that the total carbohydrate of leaves generally increased somewhat, then fell off very considerably previous to leaf fall. Total nitrogen was maximum in amount in the youngest leaves and decreased rapidly until the leaves were fully grown, then decreased more slowly. Water content was highest in the youngest leaves and decreased with age. Carbohydrates and total nitrogen of root and stem were maximum in October and November, decreased rather rapidly to a minimum in April or May, then increased from June or July to the end of the growing season.

BERTHELOT (6) has summarized a considerable number of analyses of various plants at several stages of development, but the distribution of nitrogen among its various forms was not determined.

The work of OTTO and KOOPER (24, 25), while primarily carried out with another purpose in mind, shows that there is a progressive decrease in nitrogen content of the leaves of *Syringa vulgaris*, *Sambucus nigra*, *Philadelphus coronarius*, and *Aesculus hippocastanum* throughout the season.

The literature in regard to the chemical changes in the leaves of deciduous perennials and trees is in agreement with that here cited in indicating that there is a progressive decrease in total nitrogen content of the leaf from spring to autumn, and that senescence of the leaf is preceded by a complete disappearance of nitrates and a further decrease in nitrogen, phosphorus, and potash as a result of transfer from the leaf to the stem. Concurrently with the decrease in total nitrogen there is a progressive increase in water content of the leaf, which becomes relatively stationary during the period of functional activity and declines as senescence sets in. BENEDICT (4), who has summarized the literature on the physiological changes occurring in senescence in leaves, found that old leaves of *Vitis vulpina* had materially lower capacity for absorbing water than young leaves, which he attributed to the increased content of vascular tissue in the old leaves.

Certain annual plants are known to accumulate very considerable quantities of nitrates in their stems and leaves. BOUTIN (8, 9) reported in 1873-1874 the finding of large quantities of potassium nitrate in aerial parts of *Amaranthus atropurpureus*, *A. blitum*, and *A. ruber*. PAMMEL and DOX (26) made chemical analyses and microchemical studies of *A. blitoides*, *A. graecizans*, and *A. retroflexus*, and the results of their total nitrogen determinations ranged between 1.88 and 2.49 per cent. As the quantities of protein indicated by the microchemical tests were in all cases very small, much the larger portion of the total nitrogen was non-protein nitrogen, and a considerable part may have been present as nitrate. These reports prompted further work by WOO (39) and its subsequent extension by CAMPBELL (12).

WOO made chemical analyses of roots, stems, branches, and leaves of *Amaranthus retroflexus* at three stages of growth, beginning at emergence from the ground and ending just before blooming. CAMPBELL (12) continued the work to include the mature stage and also made analyses of 25 other weed species at three stages of development. WOO had found a pronounced accumulation of nitrate nitrogen in the stems and branches with less marked increase in the roots just prior to blooming, which had entirely disappeared from all parts in the mature plant. CAMPBELL found in all the species studied that a somewhat analogous accumulation of nitrate nitrogen occurred in the early stages of growth, reaching its maximum just before blooming, then disappearing in the mature stage. Of the various orders and families represented, representatives of Chenopodiales showed the highest accumulation of nitrate, with Polygonales ranking next (represented by *Rumex crispus*, *Polygonum pennsylvanicum*, and *P. convolvulus*). When grown in soils very high in available nitrate content, as on piles of stable manure or decayed hog hair, *Amaranthus retroflexus* and

Atriplex patula showed a considerable content of nitrate nitrogen at full maturity. In all the forms used the total nitrogen content of stems, roots, and leaves decreased from the earliest stage to maturity.

Woo found that total carbohydrates, polysaccharides, and non-reducing sugars in roots and stems increased in the first two weeks after emerging from the soil, meanwhile decreasing in the leaves. Just before blooming the relations were reversed, a sharp decrease in these constituents in the root and stem accompanying the increase in the leaves. Total carbohydrate and total nitrogen showed reciprocal fluctuations in each of the three parts, increase in total nitrogen accompanying decrease in total carbohydrate and *vice versa*.

From the results reported by CAMPBELL, it would appear that accumulation of considerable quantities of nitrate in the leaves of annuals, biennials, and perennials whose above-ground portions mature and die before frost is of rather general occurrence in the period prior to blooming; but that the appearance of flowers and seed is accompanied by total or nearly total disappearance of nitrates from leaves and stems.

The work of SCHERTZ (30) on mottling of leaves of *Coleus*, taken in conjunction with the earlier work of SAMPSON (29), on abscission in the same plant, is of interest in this connection. The normal green leaves of this plant have a somewhat high content of nitrate at all stages of growth up to full size, no material reduction in nitrate content occurring so long as the leaf is functional. Leaves in which mottling had become perceptible were always lower in all forms of nitrogen (and especially in nitrate nitrogen) than normal green leaves; their phosphate content was only one-third that of normal leaves. In leaves in which the abscission layer was in process of formation, SAMPSON'S analytical data indicate that there is a reduction in nitrate nitrogen in the leaf blade with a considerable increase in the immediate neighborhood of the abscission layer previous to leaf fall. There was little evidence of any general transport of carbohydrates, phosphates, or organic nitrogen compounds out of the leaf previous to its fall. SAMPSON gives no indication as to the age of his plants nor as to whether they had reached the flowering stage. The photographs accompanying the paper of SCHERTZ (30, figs. 5, 6) indicate that the plants from which leaves were taken were producing flowers. It would consequently appear that in *Coleus* no such reduction of nitrate content of the leaves occurs at flowering as was found by CAMPBELL in *Amaranthus* and other weeds.

NIGHTINGALE (23) concludes that "nitrates may be stored by the plant within itself until the proper conditions arise for synthesis to other forms of nitrogen." This is unquestionably true. The high nitrate content of young plants and the lower content of the mature plants examined by CAMPBELL may be due to exhaustion of available nitrates within reach of

the root system and to utilization of the stored nitrate in the construction of protein, or to the possession of high nitrate-absorbing capacity by the young plant and cessation of such absorption, regardless of supply, as its utilization in growth ceases after production of flowers and seeds.

Summarizing, it would appear that in annuals, nitrates are rather generally accumulated during the period of most active growth and exhausted during development of the reproductive structures.

Experimentation

The purpose of this paper is to record some of the findings in regard to the biochemical changes occurring in the rhubarb plant in the course of its seasonal growth. Particular attention has been directed to the changes in acidity, carbohydrate, and nitrogenous constituents occurring incidental to growth, maturity, and senescence of the aerial parts, and to the effects of the annual march of seasonal conditions upon the character of these changes.

SOURCE OF MATERIAL

The material used in these studies of rhubarb was supplied through the kindness of D. N. SHOEMAKER and was grown in the variety test plot at Arlington Experimental Farm near Rosslyn, Va. The variety, Ruby, was originated at the Central Experimental Farm, Ottawa, Canada, and was named and distributed for trial by Dominion Horticulturist W. T. MACOUN (20) in 1923. The planting was made in the spring of 1927 with material obtained by the usual method of dividing the crowns of old plants. The samples were taken in 1928, so that the planting was one year old. Generous applications of well rotted stable manure were made in 1927, but no manure or commercial fertilizer was applied in 1928. The plot was frequently cultivated throughout the season to kill weeds. No flower stalks developed upon any of the plants during the season. The variety is one that does not readily form flower stalks.

METHODS OF SAMPLING

Four series of samples were taken during the season. The first of these series differed from the others in that it consisted of the first leaves which were developed by the plants in the spring. The plot was inspected on April 21, at which time the leaves were just appearing above ground. Identifying tags were attached to a considerable number of leaves having petioles 2-4 inches in length with the leaf blade not yet completely expanded. These leaves were arbitrarily assumed to be 10 days of age at this time. From the group of leaves so marked, samples were taken at intervals of 10 to 14 days, beginning April 21, up to June 15, at which time the leaves first marked were 65 days of age and were yellowing and dying.

From April 21 onward the plants were inspected at intervals of 5 to 8 days, and a number of developing leaves having petioles 2-4 inches long were tagged at each inspection. This made it possible to collect at one time a series of samples of leaves and petioles of known and varying ages up to senescence. Such collections were made on three dates, June 5, July 6, and October 17, each collection consisting of samples ranging in age from 5 to 8 days up to 60 to 65 days. In consequence, while the first series consisted of petioles and leaves all of which began their development at one time, the later series were made up of material beginning development at various dates throughout the entire period of active growth, from April 11 to October 9.

The samples were in all cases collected about 10 o'clock in the morning, after dew, if present, had evaporated. Each sample was made up of about 40 leaves from 20 plants, collected by pulling the petioles away from the crown, as is customary in harvesting for market. The material was immediately transferred to the laboratory and sampled for analysis as rapidly as possible, being protected from evaporation meanwhile. In taking the samples of leaf blades, the midrib and its lateral branches were removed and discarded, the sample consisting of mesophyll tissue with only the smaller veins, taken from various regions of a great number of leaves. The samples of petioles were prepared by cutting thin sections from upper, middle, and basal regions of each of a sufficient number of petioles to make up the desired quantity. Duplicate 100-gm. samples were taken in all cases. In taking the root samples, an entire plant was taken up, brought into the laboratory, and portions of the root system selected which were as nearly representative of the entire system as a whole as possible. As it was not possible to collect the very fine rootlets, the samples consisted chiefly of the thickened rootstocks and roots in which storage of starch and other substances occurs. The first root sample was taken April 21, at the outset of seasonal activities; the others were taken at the same time as the petiole and leaf samples.

METHODS OF ANALYSIS

The samples were preserved in sufficient 90 per cent. alcohol to make the final concentration 75 per cent. and were heated to boiling to stop enzyme action. The following November the samples were extracted with alcohol, the extracts made up to volume, and aliquots taken for determination of soluble solids, sugars, tannins, and nitrate nitrogen. Acid-hydrolyzable polysaccharides were determined upon a portion of the insoluble residue. Total nitrogen determinations were made upon portions of material dried in an air oven at 70° C. and finished in a vacuum oven.

SUGARS.—Determinations were made before and after hydrolysis with HCl at 70° F. by the MUNSON-WALKER method, the cuprous oxide being

determined by the volumetric permanganate method. The results for free reducing sugars are omitted from the tables.

TITRATABLE ACIDITY.—Titrations were made with N/10 NaOH against phenolphthalein and the results expressed as malic acid.

TANNINS.—Titrations were made with N/20 KMnO_4 with indigo-carmin as indicator.

ACID-HYDROLYZABLE POLYSACCHARIDES.—The official method of the Association of Official Agricultural Chemists for determination of starch by direct acid hydrolysis was employed. (See footnote, p. 462.)

TOTAL NITROGEN.—Determinations were made by the KJELDAHL method as modified to include nitrate nitrogen.

NITRATES.—The method recommended by the Official Methods for determination of nitrates in meats was followed.

AMINO ACIDS.—Determinations were made by the VAN SLYKE method upon aliquots of the material after preservation in alcohol. In view of the work of WEBSTER (37), it is doubtful whether the results can be considered reliable, but they may have a certain degree of comparative value.

WEATHER CONDITIONS DURING SAMPLING PERIOD

The weather conditions for the 12 months of 1928 are given in table I, which summarizes for each month the rainfall, normal mean temperature, and percentage of the possible sunshine, with departures from the respective monthly normals. The year was somewhat warmer than normal up to April 1, with somewhat deficient rainfall. April had a moderate excess of rain and was below normal in temperature; May was very close to normal in all respects; June and July were deficient in rainfall and drought conditions had become well established by August 11 and 12, at which time rainfall of more than 8 inches occurred, followed by several further rains which brought the total for the month up to 14.41 inches. September was markedly cool and deficient in sunshine; October was dry and warm, as were the following months.

In table II the weather conditions for 40 days preceding the taking of each sample are stated by periods of 10 days each, mean temperature, percentage of possible sunshine, and rainfall having been calculated from the daily records of the Weather Bureau. Certain facts are immediately obvious from inspection of the table.

The first series of samples began development about April 10 and were collected at various times up to June 15. The second series began development at various times between April 21 and June 1 and were taken all at one time on June 5. The period from April 21 to June 15 was one of progressively rising temperature, with sunshine close to the normal and with no marked deficiencies in rainfall. The 10-day period ending April

TABLE I
MONTHLY PRECIPITATION, MEAN TEMPERATURE, AND PERCENTAGE OF POSSIBLE SUNSHINE, WITH NORMALS AND DEPARTURES THEREFROM,
FOR THE YEAR 1928; WASHINGTON, D. C.

MONTH	RAIN <i>inches</i>	DIFFERENCE		TEMPERATURE <i>°F.</i>	NORMAL <i>°F.</i>	DIFFERENCE <i>°F.</i>	SUNSHINE <i>per cent.</i>	NORMAL <i>per cent.</i>	DIFFERENCE <i>per cent.</i>
		NORMAL <i>inches</i>	<i>inches</i>						
Jan.	2.63	3.38	- 0.75	35.9	33.4	+ 2.5	65	46	+ 19
Feb.	2.69	3.42	- 0.73	37.7	35.3	+ 2.4	58	54	+ 4
Mar.	2.17	3.85	- 1.68	44.1	42.6	+ 1.5	60	51	+ 9
April	4.49	3.25	+ 1.24	51.9	53.3	- 1.4	55	58	- 3
May	4.00	3.83	+ 0.17	63.1	63.7	- 0.6	62	61	+ 1
June	2.66	4.13	- 1.47	71.0	72.2	- 1.2	53	62	- 9
July	2.17	4.71	- 2.54	78.2	76.7	+ 1.5	66	64	+ 2
Aug.	14.41	4.01	+ 10.40	77.0	75.0	+ 2.0	49	61	- 12
Sept.	4.29	3.24	+ 1.05	64.9	68.1	- 3.2	43	63	- 20
Oct.	0.67	2.84	- 2.17	59.9	57.4	+ 2.5	61	61	- 0
Nov.	2.00	2.37	- 0.37	49.4	45.2	+ 4.2	48	56	- 8
Dec.	1.21	3.32	- 2.11	39.2	36.6	+ 2.6	58	51	+ 7

TABLE II
WEATHER CONDITIONS PRECEDING THE TAKING OF SAMPLES BY 10-DAY PERIODS

SAMPLING	1ST 10 DAYS PRECEDING SAMPLING			2ND 10 DAYS PRECEDING SAMPLING			3RD 10 DAYS PRECEDING SAMPLING			4TH 10 DAYS PRECEDING SAMPLING		
	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN
	°F.	per cent.	inches	°F.	per cent.	inches	°F.	per cent.	inches	°F.	per cent.	inches
Series I:												
April 21	51.4	57.9	0.24	55.1	61.3	1.42	52.3	59.9	0.35	52.9	59.7	2.01
May 5	58.3	66.4	1.96	49.7	54.9	1.11	53.9	48.3	1.32	53.9	56.5	4.39
May 19	64.2	70.6	1.01	61.6	57.2	1.28	48.5	44.3	3.02	58.1	57.4	5.31
May 26	65.5	45.4	2.54	58.7	72.7	1.35	59.1	57.2	2.00	61.1	61.3	3.72
June 5	65.6	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
June 15	69.9	56.8	0.49	64.6	54.0	0.37	65.5	46.4	2.54	66.6	52.4	3.40
Series II:												
June 5	65.5	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
Series III:												
July 6	76.3	75.7	0.55	71.8	42.9	1.25	69.2	60.2	0.49	72.4	59.6	2.29
Series IV:												
Oct. 17	67.0	61.1	0.00	59.8	51.4	0.55	74.0	64.7	0.00	67.0	59.1	0.55

21 had only 0.24 inch rainfall, but the preceding 10 days had 1.42 inches. The period May 26 to June 15 had only 0.86 inch, but the preceding 10-day period had 2.54 inches. In no case during the taking of these samples was there a period of drought such that the soil became markedly deficient in moisture, nor was there any such marked or prolonged departure from normal in temperature or in incidence of sunshine as might be expected appreciably to affect the rate of development of the plants.

The third series, collected July 6, presents some contrasts with the second series. The mean temperature for the 30 days preceding July 6 was 9 degrees higher than for the corresponding period ending June 5, while the rainfall was only 54 per cent. of the amount for that period. These samples consequently developed in a period that combined high temperatures and resulting maximum transpiration with a considerable deficiency in water supply. The fourth series, collected October 17, developed in a period of markedly deficient precipitation. The 10 days immediately preceding sampling were entirely rainless, the second 10 days had 0.55 inch, and the third 10-day period was again rainless. The dry period had been of sufficient duration at the time of sampling to reduce soil moisture to a very low level, and the mean temperature for the month preceding sampling was only 5.4 degrees below that for the third series and 3.7 degrees above that of the second series.

Summarizing, the first and second series developed in a period of ample rainfall and low mean temperature; the third series developed in a period of subnormal rainfall and maximum mean temperature; the fourth series developed in a period of markedly deficient rainfall and intermediate mean temperature. The percentage of possible sunshine is markedly uniform in all the series. Obviously, any effect of environmental conditions apparent in comparing the several series may be expected to be that of progressive reduction of moisture content of the soil accompanying the progress of the season.

Analytical results

The results of the analyses are assembled in table III, and the changes in the several constituents are graphically presented in figures 1 to 5. The general course of the changes in composition of the petiole and leaf will be indicated, after which the details of differences between the several series will be discussed.

TOTAL SOLIDS

It is noted at once that there was a very striking difference in total solids between the mesophyll tissue and the petioles. The total solids of the leaf tissue were everywhere much greater, in the early stages about three times as great, as in the petiole. Total solids of the leaf decreased

TABLE III
COMPOSITION OF RHUBARB PETIOLES AND LEAVES AT DIFFERENT STAGES OF DEVELOPMENT IN PERCENTAGE OF FRESH GREEN WEIGHT

DATE OF SAMPLING	AGE IN DAYS	SIZE OF LEAVES IN INCHES		TOTAL SOLIDS		SOLIDS SOLUBLE IN ALCOHOL		ALCOHOL-INSOLUBLE RESIDUE		TOTAL SUGAR		
		LENGTH PETIOLE	WIDTH LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	
Series I:												
April 21	10	2-4	3-4	5.21	15.49	2.96	5.64	2.25	9.85	0.65	0.74	
May 5	24	10-12	8-10	4.80	12.97	2.44	4.52	2.36	8.45	0.30	0.62	
May 19	38	12-14	10-12	5.71	10.02	2.80	3.08	2.91	6.94	0.42	0.42	
May 26	45	14-18	12-14	5.87	9.71	2.90	3.32	2.97	6.39	0.60	0.47	
June 5	55	14-18	12-14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56	
June 15	65	14-18	12-14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48	
Series II:												
June 5	5	3-4	3-4	5.33	16.40	2.84	5.60	2.49	10.80	0.40	0.89	
"	8	6-10	5-7	4.83	14.63	2.60	5.40	2.23	9.23	0.39	0.70	
"	11	10-12	6-8	4.60	14.59	2.48	4.84	2.12	9.75	0.33	0.62	
"	15	12-14	8-9	4.84	12.65	2.72	4.08	2.12	8.59	0.39	0.62	
"	19	14-16	8-10	4.56	13.27	2.56	4.16	2.00	9.11	0.29	0.40	
"	25	16-18	14-16	5.25	12.74	2.96	3.74	2.28	9.00	0.52	0.40	
"	32	18-21	14-16	5.81	11.17	3.16	3.28	2.65	7.89	0.60	0.65	
"	40	18-21	14-16	6.17	11.62	3.37	3.32	2.80	8.30	0.90	0.51	
"	55	14-18	12-14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56	
"	65	14-18	12-14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48	
Series III:												
July 6	5	4-6	3-4	6.57	18.07	3.44	5.80	3.13	12.27	0.40	0.79	
"	6	10-12	5-7	6.45	17.59	3.44	6.28	3.01	11.31	0.50	0.71	
"	13	12-16	7-10	6.15	15.82	3.20	5.40	2.95	10.32	0.60	0.74	
"	28	18-24	18-22	7.19	13.04	3.60	4.16	3.59	9.88	1.05	0.61	
"	42	20-28	17-22	7.43	12.37	3.48	3.44	3.95	8.93	0.91	0.52	
"	60	20-28	17-22	6.82	10.06	2.96	2.76	3.86	7.30	0.56	0.42	
Series IV:												
Oct. 17	8	9-14	7-10	6.29	15.57	3.56	5.32	2.73	10.25	1.04	0.74	
"	12	14-18	10-12	6.35	15.02	3.44	5.32	2.91	9.70	1.04	0.66	
"	24	16-20	10-14	7.22	13.34	3.76	4.32	3.46	9.02	1.14	0.61	
"	40	16-20	10-14	7.23	13.33	3.84	4.24	3.39	9.09	1.05	1.36	
"	60	16-20	10-14	5.64	10.00	2.52	3.20	3.12	6.80	0.58	0.61	
Root												
April 21				13.14		5.96		7.18		1.76		
May 19				12.08		5.68		6.40		1.14		
July 6				22.09		6.76		15.33		2.44		
Oct 17				24.44		6.99		18.45		2.69		

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TABLE III—(Continued)

ACID-HYDROLYZABLE POLYSACCHARIDES		ACID AS MALIC		TANNIN		NITRATE NITROGEN		AMINO NITROGEN		TOTAL NITROGEN	
PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF
0.55	0.90	0.999	0.441	0.096	1.08	0.014	0.002	0.045	0.042	0.211	0.943
0.53	0.79	0.948	0.688	0.058	0.432	0.030	0.004	0.029	0.039	0.203	0.824
0.67	0.69	0.884	0.595	0.086	0.277	0.067	0.007	0.027	0.032	0.178	0.560
0.68	0.59	1.000	0.611	0.109	0.326	0.065	0.007	0.036	0.038	0.155	0.409
0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
0.70	0.62	0.749	0.540	0.110	0.286	0.100	0.017	0.020	0.035	0.130	0.435
0.54	0.95	1.080	0.593	0.122	0.774	0.024	0.003	0.043	0.062	0.198	0.964
0.48	0.89	1.110	0.652	0.109	0.581	0.029	0.005	0.045	0.061	0.160	0.912
0.46	1.05	1.080	0.639	0.127	0.487	0.030	0.005	0.055	0.054	0.136	0.895
0.47	1.06	1.110	0.748	0.096	0.338	0.032	0.005	0.040	0.032	0.142	0.813
0.42	1.21	1.070	0.785	0.088	0.348	0.035	0.005	0.039	0.050	0.149	0.741
0.45	1.06	1.085	0.704	0.091	0.335	0.038	0.006	0.030	0.038	0.127	0.727
0.92	0.58	1.140	0.715	0.087	0.249	0.044	0.009	0.030	0.028	0.134	0.628
0.72	0.99	1.160	0.713	0.079	0.282	0.056	0.009	0.026	0.032	0.130	0.531
0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
0.70	0.62	0.749	0.540	0.110	0.236	0.099	0.017	0.020	0.035	0.130	0.435
0.76	1.06	1.520	0.749	0.168	1.222	0.017	0.002	0.071	0.093	0.267	1.090
0.79	1.30	1.580	0.821	0.153	0.975	0.022	0.002	0.068	0.101	0.228	0.944
0.77	1.78	1.380	0.799	0.123	0.455	0.026	0.003	0.057	0.065	0.197	0.867
0.84	1.10	1.320	0.843	0.201	0.437	0.027	0.003	0.043	0.058	0.149	0.662
0.78	0.81	1.150	0.628	0.162	0.290	0.053	0.005	0.038	0.055	0.148	0.594
0.75	0.75	0.781	0.493	0.265	0.265	0.073	0.010	0.010	0.043	0.121	0.316
0.63	0.98	1.560	0.893	0.111	0.359	0.023	0.003	0.070	0.066	0.201	0.807
0.64	0.84	1.430	0.816	0.114	0.385	0.026	0.003	0.070	0.055	0.186	0.734
0.74	0.90	1.300	0.876	0.110	0.392	0.026	0.003	0.050	0.072	0.180	0.675
0.72	0.82	1.280	0.950	0.115	0.385	0.030	0.004	0.041	0.041	0.159	0.635
0.67	0.65	0.934	0.680	0.100	0.331	0.045	0.005	0.039	0.046	0.132	0.367
2.24		0.362		1.260	Root	0.0082		0.096		0.417	
1.91		0.717		1.410		0.027		0.069		0.386	
10.41		0.560		1.460		0.022		0.054		0.279	
12.60		0.576		1.500		0.026		0.062		0.447	

progressively with increasing age until the leaf had practically ceased to grow, then fell off more slowly until the leaf was senescent (fig. 1, curves $A'-D'$). The whole course of development of the leaf is accompanied by a progressive hydration which varies in rate with the amount of external water supply, as evidenced by the flatness of curve D' (representing a series grown under conditions of rather severe drought). It would appear that the developing leaf is high in its content of hydrophilic colloids and that these become progressively saturated as the leaf ages. With attainment of full size and cessation of the formation of new colloidal materials, absorption of water slows down, but continues, except in one case (figure 1, A'), up to the final sampling. It seems possible that the old leaf may die because it can no longer obtain water, lacking a saturation deficit of sufficient magnitude to maintain a water balance against transpiration.

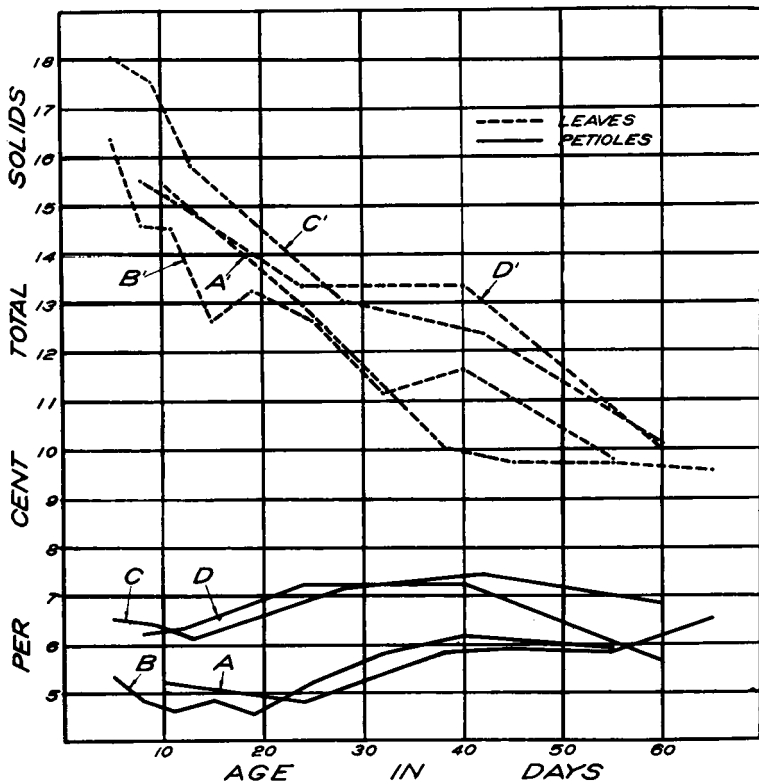


FIG. 1. Changes in total solids content in leaf blades and in petioles of rhubarb with increase in age and advance of season: A, A' , series collected April 21 to June 15; B, B' , series collected June 5; C, C' , series collected July 6; D, D' , series collected October 17.

The changes in total solids content of the petioles take a definite course which is of interest (fig. 1, *A-D*). During the period of rapid elongation of the petiole the solids decrease. As elongation slows down and the period of maximum photosynthetic activity of the leaf is entered upon, solids increase again, becoming stationary or falling off somewhat as senility of the leaf commences.

It will be observed that there is considerable difference in total solids content of both leaves and petioles in the series taken at different seasons. In series I and II, developing in the spring, total solids are consistently lower at all stages in both leaves and petioles than in series III and IV, collected in July and October. Further, series IV, collected in October, is distinctly higher in solids than series III, except in the old petioles. These differences would appear to be explained by the fact that the water supplied by rainfall during the last month of development of series III and IV was only about one-half and one-eighth that received in the corresponding period by series I and II. It would appear that the saturation deficit of the leaf tissues is largely satisfied in the first half of the life period if the water supplied the root is ample, but that its satisfaction is progressively retarded with progressive decrease in water supply.

Total solids of the root show a decrease from the first sampling, April 21, to that on May 19, due to depletion of the reserves of the root system by the developing foliage. On July 6, storage of reserves is well advanced, and on October 17 the total solids content of the roots is more than 200 per cent. of that present at the height of the growing period on May 19.

ALCOHOL-SOLUBLE SOLIDS

The alcohol-soluble solids of the leaf blade are high in the earliest samples taken and decrease rapidly until the leaf is full-grown, then more slowly up to old age. The final value in old leaves is about 50 per cent. of that of the youngest samples. In the petioles, soluble solids fluctuate within narrow limits, decreasing somewhat in the period of most rapid growth, then returning to the original level. In series III and IV there is a decrease in the final sample not found in the earlier series, for which no explanation is apparent.

The general level of alcohol-soluble solids is higher in the petioles of series III and IV, but there is no consistent difference in the leaves. The alcohol-soluble solids of the root show little change, decreasing slightly from April to May, then rising to somewhat higher levels in July and October.

ALCOHOL-INSOLUBLE SOLIDS

The alcohol-insoluble solids of the leaf blade decrease rather rapidly until the leaf is full-grown, then more slowly, with some fluctuations, to

a value at final sampling about two-thirds of that found in the earliest sample. The alcohol-insoluble fraction in the petioles remains stationary or decreases during rapid growth, then rises somewhat with increasing age. The general level of insoluble solids in the petioles is a little higher in series III and IV than in the earlier series.

The fluctuations in both alcohol-soluble and alcohol-insoluble fractions in petioles and leaves of series I suggest that both may be temporarily affected by climatic conditions, and particularly by rainfall.

Alcohol-insoluble solids in the roots decrease to a minimum in May, when rapid development of aerial parts at the expense of stored reserves is occurring, then increase rapidly to the July sampling, and more slowly to October, when the amount is 282 per cent. of that present in mid-May. The large seasonal change in total solids of the roots is practically wholly due to the change in amount of the alcohol-insoluble fraction.

SUGARS

It is obvious that the total sugars are low in both leaf and petiole, and the differences between them are not marked. There is a slight tendency for the sugars of the leaf blades to be highest in young samples and lowest in old samples, while lowest in young petioles and higher in older ones; but between the two extremes there is considerable and rather abrupt fluctuation, so that the curves are irregular. It does not seem to be justifiable to draw any very definite conclusion as to the mechanism of the transportation and the use that is made of the sugars.

ACID-HYDROLYZABLE SUBSTANCES (POLYSACCHARIDES)¹

The acid-hydrolyzable substances are very low in both the leaf and petiole. The figures indicate that the change in content with age is not great. They seem to be highest in both petioles and leaves in series III. In the series taken in June and July these substances are highest in the samples of medium age, being low when young and decreasing again with age. This is not apparent in the other two series. Starch is practically absent in the petiole and not high in the leaf. Acid-hydrolyzable substances are at a minimum in the root in May, increasing to over 600 per cent. of

¹ The term "acid-hydrolyzable polysaccharides" as applied to the substances converted to reducing sugars by the official method of direct acid hydrolysis for starch of the Association of Official Agricultural Chemists, which was here used, is technically inaccurate, since the reducing sugars obtained may be derived from polysaccharides, pentosans, glucosides, nucleic acids, or other substances having a sugar group in the molecule. While a misnomer, it has the merit that prolonged usage has made biochemists familiar with its real meaning as here employed. "Acid-hydrolyzable substances," while less restrictive, is indefinite unless the exact conditions of the hydrolysis are stated.

the spring value in October. Approximately one-half of the total solids of the October sample is in this fraction.

TANNIN

There is a striking difference in the tannin content of the leaf and petiole, that of the leaf being one and one-half to four times as high as that of the petiole. In the petiole the stage of maturity makes no striking difference until the very old samples are reached, when it becomes much lower. In the leaf the tannin is highest in the young and lowest in the old material. The decrease in amount in the leaf is rapid in the period of most rapid growth, 5 to 20 days of age, then becomes slow and fluctuates irregularly. Tannins in the root increase slightly from first to last sampling.

TITRATABLE ACIDITY

The titratable acidities of petioles and leaf blades show consistent and striking differences at all stages (fig. 2). That of the petiole is always much higher, being double that of the blades in the youngest samples and about one and one-half times that of the blades in the older ones. The changes

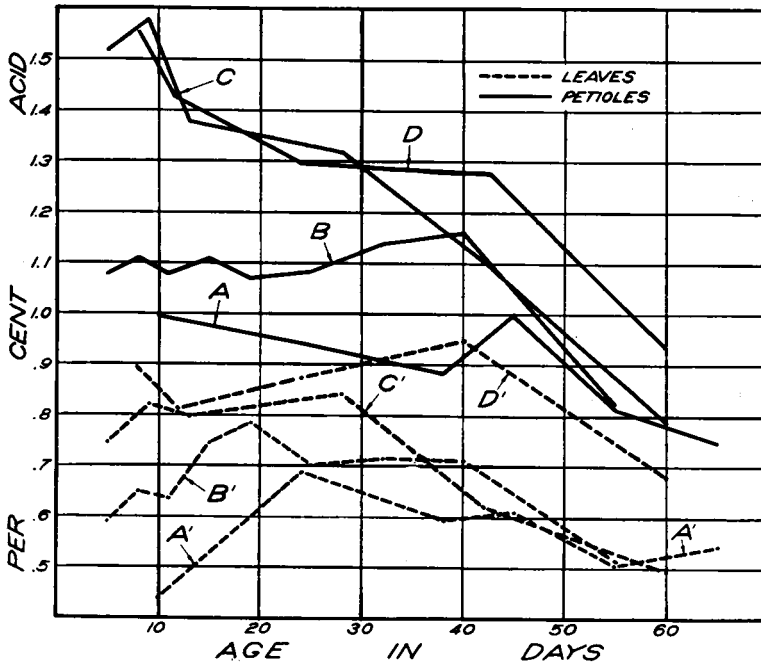


FIG. 2. Changes in titratable acidity of leaf blades and petioles of rhubarb with increase in age and advance of season: A, A', series collected April 21 to June 15; B, B', series collected June 5; C, C', series collected July 6; D, D', series collected October 17.

with age in the leaf tissue are an increase in the period of rapid growth, a period of little change during the period of maximum photosynthetic activity of the leaf, and a gradual decline as the leaf becomes old. In the petiole, acidity remains somewhat constant or decreases only slightly until the petioles are 45–55 days old, then declines rather rapidly in the old samples. It is noteworthy that the acidity of both petioles and blades of series III and IV is considerably higher throughout than in series I and II, that of the petioles being about 150 per cent. This difference may find its explanation in the reduction of available water supply during the development of the later series.

TOTAL NITROGEN

The curves representing total nitrogen content of petioles and leaves show the striking differences which exist (fig. 3). The total nitrogen of the leaf mesophyll is in the young leaf 400 to 500 per cent. of that of the petioles of like age; that of the old leaves is approximately 300 per cent. of that of old petioles. There is a marked decrease in total nitrogen of leaves with age, the final values for leaves 60 to 65 days old ranging from 35 to 45 per cent. of those of the youngest samples. The decrease is not especially rapid at any particular stage, but continues at a rather uniform rate throughout the whole life of the leaf. The petioles also decrease progressively and rather uniformly in total nitrogen content throughout life, the total decrease amounting to 30 to 50 per cent. of the initial content. The differences in level of total nitrogen content between the several series are not great in amount and are not correlated with the march of seasonal conditions.

The total nitrogen content of the roots decreases up to July 6, but has risen on October 17 to a level slightly above that of the initial sample of April 21. There is apparently a progressive depletion of total nitrogen in the roots so long as new leaves are being developed, followed by an increase after such development has ceased for the season.

There is clearly no such great and rapid reduction of total nitrogen in the old leaves by transfer into the perennial parts as has generally been found to occur in woody perennials and trees. The curve of decrease in the leaf is very nearly a straight line with no alteration in direction with old leaves, despite the fact that the blades of the final samples were generally yellowing and had dead areas at the edges. The present data do not permit decision as to whether the reduction in percentage of total nitrogen is only apparent and not actual, and attributable to the changes in composition occurring with age, or whether slight actual decrease occurs.

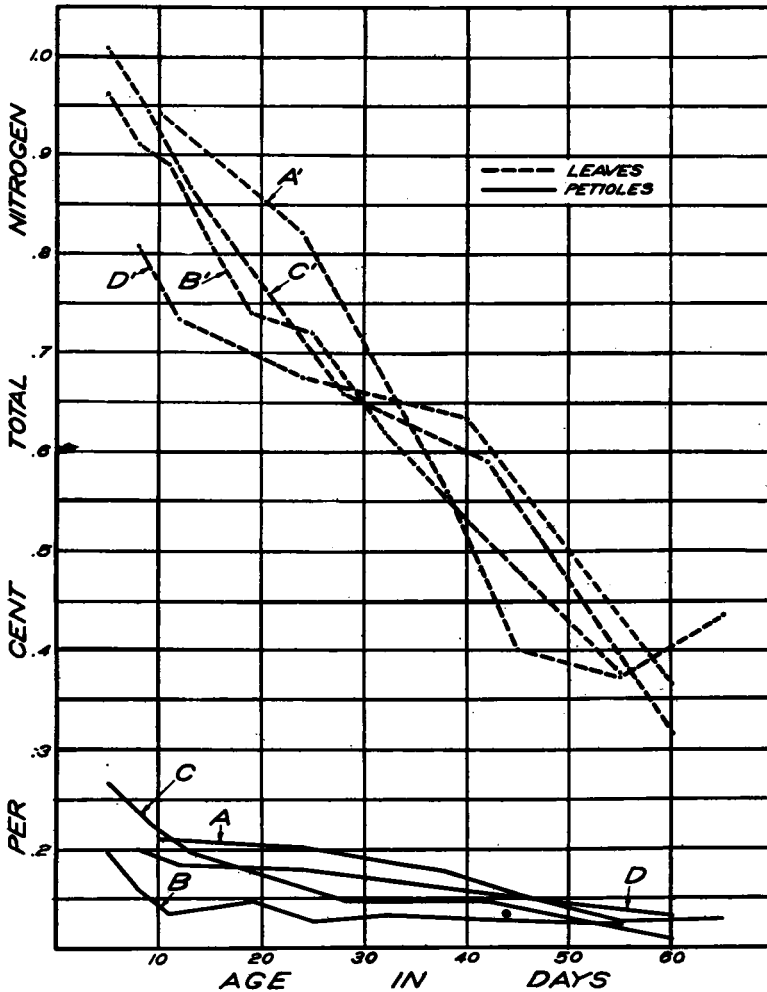


FIG. 3. Changes in total nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: A, A', series collected April 21 to June 15; B, B', series collected June 5; C, C', series collected July 6; D, D', series collected October 17.

AMINO NITROGEN

The determinations of amino nitrogen were made upon material preserved in alcohol. They were completed prior to the appearance of the paper by WEBSTER (37) on the losses of amino nitrogen occurring in material preserved in this manner, especially when nitrates are present. It is very questionable whether the results have any quantitative value. The extremely irregular character of the curves is not conducive to confidence in

their accuracy. The results are included in the table and represented in figure 4 for any comparative value they may have. They indicate that there is a progressive but rather irregular decrease in amino nitrogen from first to last samplings in both leaf blades and petioles. They seem to indicate also that there is a decided difference in general level of amino nitrogen content between the earlier and the later series, as series III and IV are

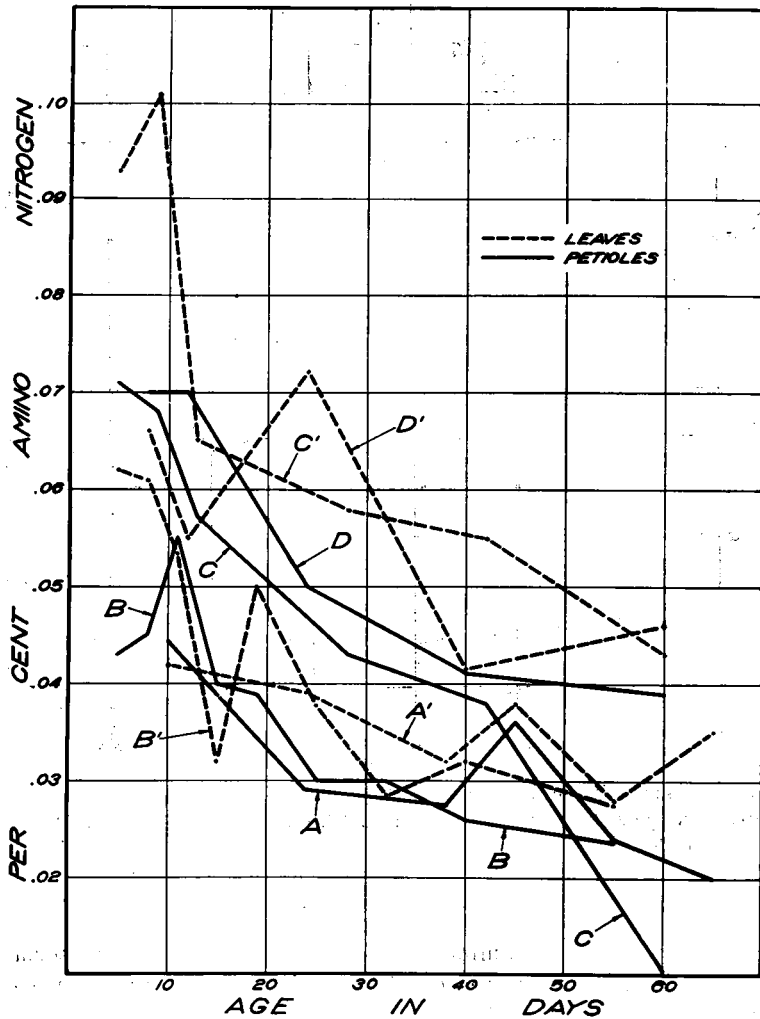


FIG. 4. Changes in amino nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A, A'*, series collected April 21 to June 15; *B, B'*, series collected June 5; *C, C'*, series collected July 5; *D, D'*, series collected October 17.

very materially higher throughout, both in petioles and leaves, than are series I and II. Whether the concurrence of higher amino nitrogen values in these series with the lower available water supply during their development is significant or merely a coincidence cannot be determined in view of the questionable character of the method employed.

NITRATE NITROGEN

As has been the case with several other constituents, and particularly with total nitrogen, there are striking differences between the leaf mesophyll and the petiole in nitrate nitrogen content. In contrast to total nitrogen, nitrate nitrogen is highest in the petioles, and the differences between petioles and leaves are greatest in the oldest material. In both petioles and leaves, nitrate nitrogen is minimum in amount in the youngest samples and rises with age (fig. 5). The amounts present in leaves are always low but the increases, while small, are consistent and significant, the final samples of the several series having amounts ranging from 160 to 850 per cent. of those of the initial samples of their respective series. The amounts initially present in the youngest petioles are much larger than in leaves, and increase rapidly with age, the final content of the old petioles ranging between six and nine times that of the old leaf tissues. It is apparent that the plant absorbs quantities of nitrate nitrogen far in excess of its anabolic requirements, and that the petioles serve as storage organs for the deposition of the excess. That this nitrate nitrogen is neither used in the leaf nor returned to the roots is clear, since the petiole attains its maximum nitrate nitrogen content when the leaf blade is yellowing and dying. The nitrate nitrogen content increases rather rapidly, even during the period of rapid growth when synthesis of protein is actively in progress, but becomes more rapid after the leaves are full-grown. There is, of course, a possibility that lack of some other constituent prevented utilization of the nitrate nitrogen and that it was consequently stored in the petioles, the metabolic processes never becoming such that it could be used. Further study will be necessary to determine whether development of flower stalks is accompanied by withdrawal of nitrate nitrogen from the petioles, since the variety employed is one which only exceptionally develops flower stalks, and none were developed during the progress of the work.

The progressive accumulation of nitrate nitrogen in the petioles is a matter of great practical importance in the canning of rhubarb, since nitrate nitrogen has an intense corrosive action upon the tin can. This matter has been dealt with in detail by CULPEPPER and MOON (14) in their report upon the behavior of rhubarb in canning.

It is unfortunate that soil samples were not taken and that it cannot be determined whether the amount of nitrate nitrogen in the tissues shows any

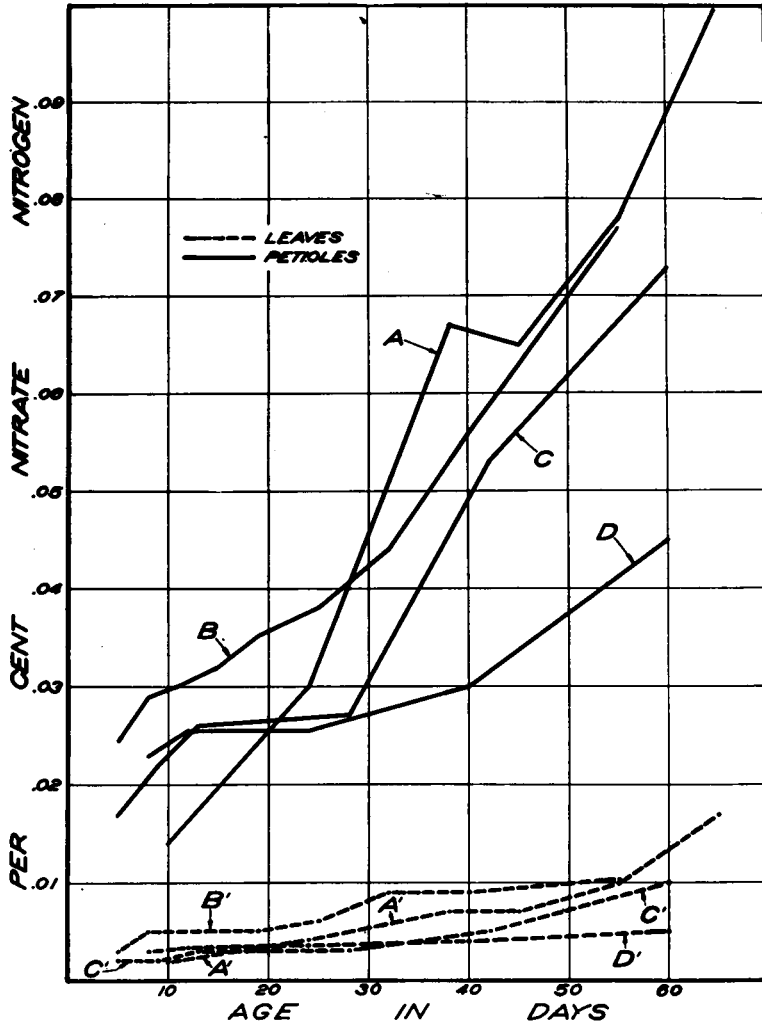


FIG. 5. Changes in nitrate nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A, A'*, series collected April 21 to June 15; *B, B'*, series collected June 5; *C, C'*, series collected July 6; *D, D'*, series collected October 17.

correlation with the amount present in the soil. It is probable that the reduction in amount of available soil nitrates with advance of the season may be responsible for the differences between the earlier series and series III and IV. In the first two series, nitrates are higher in both leaf blades and petioles than in the later series. Series III, taken in July, is consistently somewhat lower throughout in both leaves and petioles than I and

II while series IV is lower than III, the difference being especially marked in the old petioles. This may be a result of progressive decrease of available nitrate in the soil. Another fact which is significant in relation to the low nitrate content of series IV is the extraordinarily heavy rainfall of August, which had a total precipitation of 14.41 inches, or 10.40 inches' excess over the normal, largely occurring as heavy rains between August 11 and August 26. This excessive precipitation, which was followed by drought extending from mid-September through October, probably resulted in leaching out much of the nitrates present in the soil, and is without doubt a significant factor in the reduction of the nitrate content of the older samples of the October series to less than half that of the corresponding samples taken earlier in the season. That the reduction of available nitrate was limited in its effect to reducing the storage of excess nitrate in the petioles and did not affect the total nitrogen content of the leaves and petioles is immediately apparent from the graph (fig. 3, curves *D* and *D'*).

Discussion

The biochemistry of the rhubarb plant presents some interesting points of difference from that of woody perennials or that of annuals and biennials, which may be briefly summarized.

The work of LECLERC DU SABLON (15) with leaves of chestnut, pear, peach, quince, and willow trees and raspberries, and that of TUCKER and TOLLENS (34) with *Platanus occidentalis*, indicate that in these forms there is a progressive decrease in water content with age. A similar situation prevails in annuals, and is attributable in part to progressive increase in vascular elements and cutin and in part to the presence of such products of photosynthesis as starches and sugars. In the work of the investigators cited, as well as in all similar studies found in literature, the entire leaf and petiole were taken together, so that it is impossible to ascertain the nature of the changes taking place in the leaf mesophyll as distinguished from petiole, midrib, and veins.

In the rhubarb leaf from which petiole and larger veins have been separated, the situation with respect to water content is precisely the reverse of that recorded for entire leaves of other plants. The youngest material obtained had a minimum water content which increased steadily with advance in age up to senescence, but most rapidly during the period of rapid increase in size. In this respect the growth of the leaf blade of rhubarb bears less resemblance to that of other and entire leaves than it does to that of young fruits, which have been shown by CALDWELL (11) to be initially high in total solids and to increase markedly in water content during the phase of most rapid growth. The extent to which the situation

found in the leaf blade actually differs from that in the mesophyll of other types of leaves can be known only when the changes in the leaf blade proper, separated from the petiole and principal veins, have been studied in such leaves. In one respect the rhubarb leaf differs markedly from those of most other dicotyledonous plants, namely, in the practical absence of stored starch at all times.

The carbohydrate economy of the rhubarb plant presents some interesting problems. The total sugar content of the leaf is always very low, reaching 1 per cent. of the fresh weight in only one sample. It is with one exception highest in the youngest leaf blades of the series, collected while still largely folded and partially covered by the soil, and consequently before active photosynthesis could have begun. The amount present falls off slightly during the period of most rapid growth, rises as the leaf becomes full-grown, and drops again in senescence; but the changes are within extremely narrow limits and could not be considered significant did they not occur in all the series. The sugar content of the petiole is also low at all stages of growth, being sometimes above and sometimes below that of the corresponding leaf blade. In the October series it is somewhat higher throughout than in the earlier series, but drops abruptly to the same general level in the final sample. The sugar content of the root system is practically the same on April 21 and October 17, but is lower in May and higher in July.

Starch is present only in traces in petiole and leaf, as determined by microchemical tests, and the total acid-hydrolyzable substances are always low. In the leaf the amount of such substances present in the young, partially folded blade is in most cases about as great as in the fully expanded photosynthetically active leaf, and the amount present in the old, yellowing leaves is two-thirds to three-fourths that present in the active leaves. The acid-hydrolyzable substances of the petiole are lower in amount than those of the leaf blades, and fluctuate irregularly without significant change from youngest to oldest samples. There is no evidence of an accumulation of starch either in leaf or petiole as a result of photosynthetic activity, and the results indicate that there is no accumulation of sugar as such in the leaf. The amounts of sugar in the leaves and their petioles vary independently in such fashion as to make it clear that there is no concentration gradient such as has been shown to exist in various other plants. While the samples were uniformly collected at 10 A. M. on clear days, when photosynthesis should have been actively in progress for several hours, the leaves and petioles of fully functional leaves show no consistently higher content of sugar than do the young, not yet expanded leaves or the old, yellowing ones. The manufacture and transfer of photosynthetic products is accom-

plished without any such accumulation of sugars in the leaf or in the conducting system as is a normal accompaniment of photosynthesis in foliar organs. The absence of evidence for such accumulation is the more remarkable in view of the rather high rate of increase in dry weight found by SACHS (28) in *Rheum*, by MÜLLER (21) in the related *Rumex*, and by BROWN (10) in *Polygonum* when the leaves were exposed to bright illumination.

The acid-hydrolyzable substances of the roots were low when first examined on April 21, at which time the largest leaves had petioles 2-4 inches in length and had only partially unfolded their blades. The content was slightly lower a month later. On July 6 it had increased to 545 per cent. and on October 17 to 659 per cent. of the amount found on May 19. The concurrent changes in sugar content of the roots are within rather narrow limits, and the variations in total solids agree closely in direction and amount with the changes in hydrolyzable substances.

In contrast with the low concentration of sugars in petiole and leaf and the absence of a concentration gradient in sugars from leaf blade to petiole, the acid content of the leaf mesophyll is always markedly below that of the petiole. Also, the acidity of both leaf blade and petiole increases to a maximum as the leaf attains full size and declines markedly as the leaf becomes senescent. In both blade and petiole the acid content at all stages of growth equals or exceeds the sugar content, and in the petioles is often two or three times as great. The acid content of the root system fluctuates considerably, being lowest in April, increasing 100 per cent. in the next month, and then declining to a nearly constant level from July through October. At all times the acidity of the roots is below that of the leaves and half or less than half that of the petioles. Since NELSON (22) found 1.77 per cent. malic, 0.41 per cent. citric, and 0.12 per cent. oxalic acid in the petioles, the results of the acidity determinations are reported as malic acid.

In the present work, only such oxalates as are soluble in both 80 per cent. alcohol and in water are included in the determinations of titratable acidity, as the titrations were made upon aliquots of the alcoholic extract after evaporating the alcohol and taking up in water. This method includes free acids and such soluble oxalates as those of potassium, sodium, and magnesium, but does not include calcium oxalate. According to ANGERHAUSEN (1, 2), the oxalates of the leaf blade of rhubarb are largely water-soluble; those of the petiole practically wholly calcium oxalate. The data of table III indicate that total titratable acidity is at all times much higher in the petioles than in the leaf parenchyma, but give no indication as to the nature of the acid or acid salts present. No determinations of the total oxalate content of leaf blades, petioles, or roots were made.

The fact that the acid content of the young petiole and leaf is already rather high at a time when the leaves have barely emerged above ground and the blades have only partially expanded raises some interesting questions as to the rôle played by acids in the economy of the plant. CALDWELL (11) found that the period of most rapid growth in young fruits was also a period of rapid increase in both actual and titratable acidity, both of which culminated in maximum values about the time that the curve of growth passed into the self-retarding phase. His results indicate that in fruits, maximum acid production accompanies rapid cell division and increase in size, and a similar situation appears to exist in the young leaf.

Since the acid content of the mesophyll tissue is consistently lower than that of the petiole, the question arises whether acids are formed in the mesophyll directly from the products of photosynthesis and transferred into the petioles, or whether they are formed in the petioles as well as in the leaf tissue. STEINMANN (32) found that the titratable acidity of the mesophyll tissues of rhubarb increased slightly (10–15 per cent.) from apex to base of the leaf, while the increase in the veins from the smaller veinlets to the basal portion of the midrib was about 60 per cent., and a further slight increase was found in the petiole. These results STEINMANN considers as evidence that acids are formed in the leaf and transported to the roots through the petioles in the same manner as carbohydrates. The increase in acidity occurring in the leaf during a day's exposure to sunlight and the progressive decrease occurring in leaves kept in darkness in his experiments support his conclusion. His most conclusive proofs that acids are formed in the leaf and transported through the stem are derived from a series of experiments in which the petiole was cut half way through at the base of the leaf, thus severing the vessels of one-half the blade. After exposure to a day's sun, the acidity of the intact half of the leaf was always lower than that of the half with severed vessels; further, a reduction in acidity during darkness occurred in the intact half while no change occurred in the severed half. The transport of acids is consequently a constant process, in light as in darkness. STEINMANN also points out the possibility that organic acids may be not products of disintegration processes but intermediate products of constructive assimilatory processes. RUHLAND and WETZEL (27), on the other hand, consider that the progressive accumulation of acids and of ammonia and nitrate nitrogen in the plant with increasing age is the result of deamination of proteins.

ANGERHAUSEN (1, 2) states that the oxalic acid content of the leaf blades is approximately double that of the petioles, and that the oxalates of the leaf are predominantly potassium oxalate while calcium oxalate is predominant in the petiole. RUHLAND and WETZEL state that the youngest petioles contain only succinic and malic acid with scarcely a trace of oxalic,

and that later in their development there is a sharp decrease in the former concurrently with rapid increase in oxalic acid. The work of VICKERY and PUCHER (36), and that of SMIRNOW, ERYGIN, DRBOGLOW, and MASCHKOWZEN (31), indicates that the situation in tobacco is the reverse of that in rhubarb, as oxalic acid is present in the young tobacco plant but decreases as development of the plant proceeds.

BERTHELOT and ANDRÉ (5) determined the oxalic acid content in roots, stems, petioles, and leaves of *Rumex acetosa* in young, actively growing and old, fruiting plants (June 26 and September 27). In the young plant the oxalic acid content of the leaf blades exceeded that of the petioles and larger veins in the ratio of 12 to 10; in the old plants the acid content of leaf blades was lower than that of petioles and stems, the ratio being 4 to 5. The acid of the leaf blades was predominantly in soluble form in both young and old plants; that of the petioles was predominantly soluble in young plants and chiefly insoluble in old ones. The acid of the roots was wholly in insoluble form. The relative amounts present in old petioles and leaf blades were 60 and 35 per cent. respectively of those in the young parts. The situation in *Rumex* consequently differs from that found in rhubarb in that the acidity of young leaf blades is greater and that of old leaf blades less than that of petioles, while the petioles of rhubarb are always much more acid.

At the close of their resumé of the literature on the origin of organic acids in leaf tissues, VICKERY and PUCHER (36) remark that the synthesis and transformation of the organic acids in higher plants are even more obscure than is the case in the molds and yeasts. It may also be said that in no part of the field is the obscurity greater than in that relating to the rôle of oxalic acid in green plants. No generalizations seem possible, since practically every fact established by the study of one plant is definitely contradicted by the findings in regard to another plant, as instanced in the preceding paragraph. In the case of rhubarb, it seems to be clearly established by the work of STEINMANN (32) that there is a continuous production of acid, assumed by him to be oxalic acid, in the leaf blade when the leaves are exposed to light; and that translocation of the acid through the veins into the petiole occurs both in light and darkness, so that there is a concentration gradient rising from a minimum at the leaf tip through the veins and midrib to a maximum in the middle of the petiole. That soluble oxalates are transformed into insoluble form in the petiole is evidenced by ANGERHAUSEN'S results. Data here presented show that the maximum concentration of titratable acids found in leaf and petiole throughout life is found in the period of full photosynthetic efficiency, with lower concentrations in juvenile and senescent stages. These facts correlate the rate of production of acids with the general level of photosynthetic activity.

They leave the questions as to the mode of origin and rôle of oxalic acid in the plant unexplained. Whether it is a product of deamination of amino acids, as suggested by RUHLAND and WETZEL, or an intermediate product of photosynthesis playing a rôle in the metabolism of the plant analogous to that of simple sugars in other plants, as suggested by STEINMANN, or a waste product resulting from the operation of an insufficient respiratory mechanism which cannot carry the decomposition further, as the older literature implies, can be determined only by further research.

The course of the changes in nitrogenous constituents in the leaf is rather uniform in the several series. Total nitrogen and amino nitrogen are maximum in the youngest leaves and decrease rather steadily until the leaf is full-grown, after which amino nitrogen becomes fairly constant while total nitrogen undergoes further decrease until the leaf is senescent. There is rather close parallelism between the curves for the decrease in total solids and those for decrease in total nitrogen in the several series of leaf samples (figs. 1 and 4), which suggests that the reduction in amount of total nitrogen on the basis of reduction in percentage of fresh weight is due largely to dilution of the solids with water. In the initial samples of the several series, total nitrogen forms 5.8 to 6 per cent. of the total solids of the leaves; in the final samples it forms 3.15 to 4.5 per cent. of the solids. There is consequently no such large reduction in total nitrogen as SWART (33) and TUCKER and TOLLENS (34) found to occur in the leaves of *Platanus* and a number of other trees and perennial shrubs. In the case of *Platanus*, this reduction amounted to more than three-fourths of the total. The persistence of a relatively high content of total nitrogen in the old leaves does not disprove but certainly lends no support to the theory of RUHLAND and WETZEL (27), who attribute the formation of oxalic acid to the deamination of protein. Moreover, if the results for amino nitrogen are to be relied upon, its uniformly low concentration in petiole and leaf at all times would appear to militate against the possibility that deamination can be occurring at a rate sufficient to account for the large amount of oxalic acid and its salts present in the tissues.

A unique feature of the metabolism of the plant brought out by the present study is its remarkable capacity for absorbing and storing nitrates in amounts very far in excess of any subsequent needs. The work of WOO (39), CAMPBELL (12), and NIGHTINGALE (23) has made it evident that a wide variety of annuals and biennials, in their earlier stages of development, have high nitrate-absorbing capacity and store considerable amounts of nitrates in the aerial parts. Apparently high nitrate absorption is a function definitely limited to the early vegetative stages, since the plant becomes nitrate-free later in the season as flowers and seeds develop, and remains nitrate-free so long as it continues to live. In this respect, as in

some others, the rhubarb plant presents a very different situation. The young and rapidly growing petioles and leaves are low in nitrate content, its utilization in the construction of protein apparently keeping pace with absorption and transport from the roots. But with the completion of growth of the leaf there is rapid increase in nitrate content of both leaf blade and petiole, but more especially in the latter, which continues without material slackening in rate until the leaf blades are yellowing and dying. Series of leaves developed early and late in the season, hence both before and after the usual period of development of the flower stalks, behave alike in this respect. Such differences in amount of nitrates stored as occur in the earlier and later series appear to be attributable to seasonal reduction in the soil nitrate supply and in available water rather than to any alteration in the capacity of the plant for absorbing and storing nitrates. That the nitrates so accumulated are never used in the petioles or translocated elsewhere is conclusively shown by the fact that the petioles of senescent leaves have maximum amounts, the total present in one case when calculated as molecular nitrogen reaching 1.53 per cent. of the dry weight of the plant. Furthermore, the course of the changes in nitrate content of the root system, which increases from April to May and then remains constant throughout the season, shows no relation to the changes in the petioles and leaves. The nitrates of the root are not depleted by the accumulations of nitrate in the petioles of the successive series of leaves, nor is there any indication that they are increased by translocation of nitrates out of the petioles as the leaves complete their life history and die. Lastly, there is obviously no appreciable utilization of the accumulated nitrates in the formation of amino acids or protein in the mature or aging petiole, since its content of total nitrogen decreases progressively from the unfolding of the blade to old age. There was in no case any development of flower stalks, so that we are without knowledge as to what would occur to the nitrates of the petioles in the course of development of flowers and seed.

Summary

1. The biochemical changes occurring in the roots, petioles, and leaf blades of the rhubarb plant in the course of the season's growth were followed by the collection and analysis of four series of samples, each consisting of subsamples of petioles and leaf blades of varying ages from 5 days onward to senescence, at intervals during the season. The first series consisted of the first leaves developed by the plants in spring, which were collected at intervals from April 21 to June 15; the later series was obtained from leaves developing in sequence and were taken on June 5, July 6, and October 17. Samples of the roots were taken on the same dates.

2. The seasonal march of climatological conditions during the period of development and collection of the samples exerted an appreciable influence upon the rate of development of the different series, the petioles and leaf blades of the later series attaining full size in a much shorter time than did those of series I and II. The chief environmental factor to which the more rapid development of the later series is probably attributable is higher mean temperature.

3. The general composition of the several series shows some differences which appear to be attributable to another environmental factor, namely, rainfall. Series I and II developed in a period of ample rainfall very equably distributed over the whole period of growth; series III developed in a period of considerably reduced rainfall; series IV developed in a period of well established drought, the total rainfall for 30 days preceding sampling being 0.55 inch. The reduction in precipitational water supply is reflected in the lower water content of both petioles and leaves at all stages of growth in series III, developed in a period in which transpiration was maximum. The effect of drought during development is also apparent in the leaves of series IV, which retained until senescence set in the high solids content characteristic of developing leaves.

4. Total solids content was maximum in the leaf blade at the time the blades were emerging from the soil and still partially folded, decreased rapidly during growth of the leaf, and became mostly stationary after growth ceased. In the petioles there was a decrease in total solids during rapid elongation, followed by an increase which brought the solids content of the old petioles somewhat above that of the young ones.

5. The alcohol-insoluble solids of the leaf blade decreased progressively throughout the life of the blade; those of the petiole were fairly constant until growth was completed, then increased somewhat in the old petiole. Alcohol-soluble solids of the leaf blade decreased rather steadily throughout life; those of the petiole decreased during rapid growth, then rose as growth was completed.

6. Leaf mesophyll and petiole were practically starch-free at all ages. The sugar content of both was always low, that of the leaf blade being usually highest before the blade had unfolded, decreasing somewhat during growth and fluctuating within narrow limits in the full-grown leaf. Sugar content of the petiole was generally minimum during rapid growth, rising slightly in the old petiole. The fluctuations in sugar concentration in petioles and blades are independent and there was no evidence of the existence of a concentration gradient such as would be expected if large amounts of sugar were transported from the leaf through the petiole.

7. The acid-hydrolyzable polysaccharides of the leaf in two series increased slightly up to attainment of full size; in the other series there was

a slow decrease from youngest to oldest leaves. The acid-hydrolyzable material of the petiole remained stationary or increased within very narrow limits. In both parts its concentration, like that of sugars, was always low.

8. The titratable acidity of the leaf mesophyll and petiole increased during rapid growth, then declined somewhat in the older stages. The acidity of the petioles was at all ages 150 to 250 per cent. that of the leaf blade. In some cases titratable acids, calculated as malic, made up 18 to 25 per cent. of the dry weight of the petiole.

9. The total nitrogen content of leaf blades and petioles declined gradually from youngest to oldest samples in every series. The reduction was relatively much less than that reported for the leaves of woody perennials and trees.

10. Nitrate nitrogen was present in extremely small amounts in the young leaves and increased considerably after growth was completed and the leaves became old. The youngest petioles had seven to eight times as much nitrates as the corresponding leaf blades and increased steadily to a maximum in the senescent petioles, which might have a nitrate nitrogen content equivalent to 1.53 per cent. of the dry weight. There was no indication that this nitrate is used or translocated elsewhere.

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