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THE RELATION OF STORED FOOD TO CAMBIAL ACTIVITY IN THE APPLE

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INTRODUCTION

This paper reports an attempt to determine, by defoliation methods, to what extent cambial activity and storage of food are correlated. There have been observations along this line for many years, which have been ably summarized by Knudson,[7] Harper,[9] Grossenbacher,[3] André,[1] and Robbins.[11] The present state of knowledge, as judged from these and other less intimately related papers, seems to be a well-defined idea that cambial activity is dependent on leaf activity.

Wieler[14] found that covering three or four-year-old trees of *Quercus sessiliflora* before leaf development in spring, by means of boxes lined with black paper, caused a reduction in diameter growth as compared with checks. Jost (cited by Grossenbacher[3]), removed the terminal buds and thereby caused failure of radial growth in pine. Harper[4] observed the effects of successive defoliations of the larch by insects which caused first, a reduction in thickness of the cell walls and then, a decrease in the width of the annual rings until, in a few years, growth ceased entirely.

A report by Harvey[6] which is closely allied, and in some respects very similar, to the paper here presented, has recently appeared. Defoliation was found to accelerate or retard growth as measured by terminal elongation, according to the stage of development of the shoot. This criterion of growth makes direct comparison difficult

* Also presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

between Harvey's work and that here reported. There may not be a particularly good correlation between terminal and lateral growth. (See tables 8 and 9 below.) Differences in methods and materials also may account for the somewhat different results obtained. However, the hypothesis he developed for the unification of his results, that is, the carbohydrate-nitrogen ratio, should be generally applicable. This phase will receive attention later in this paper under Discussion and Conclusions.

The above citations include the chief contributions having an immediate bearing on the present problem. With the exception of the work of Harvey, the results are practically all "qualitative" in character. Quantitative data on the checking of diameter increase by defoliation were considered necessary as a foundation for any further development of the facts involved in this phenomenon, particularly with regard to change in composition that might prove to be associated with it.

MATERIALS AND METHODS

For the purpose of this investigation, trees were selected in the spring of 1922 from a six-year-old McIntosh orchard at the New York Agricultural Experiment Station, at Cornell University, Ithaca, New York. Because of the youth of the trees, there was no fruit production to interfere with vegetative relationships. Four of the trees had all of their leaves removed as soon as they appeared in the spring, that is on May 3. As new leaves appeared subsequently, they were also removed. Another group of four trees were allowed to grow normally until June 6, when they also were defoliated and kept bare as in the previous series. Four other trees were half defoliated, i.e., had all the leaves from half of the tree removed, on the same dates as the whole trees. Material was collected from these trees at intervals of from one to two weeks from the latter part of April until September 8, 1922, and consisted usually of ten one-year-old twigs from each tree.

Portions of each twig were taken from the apical, middle and basal regions, each about 1 cm. in length. The remainder of each twig was prepared for analysis as described below. These 1 cm. pieces were killed in 50 per cent alcohol. The alcohol was removed by washing in water, and the pieces were then treated with hydrofluoric acid for two weeks. The acid was then washed out with water, and the pieces placed in glycerin and alcohol (one-half glycerin, one-half 95 per cent alcohol, by volume). They were sectioned from this mixture, without imbedding. It was found that sections twenty-five to thirty-five microns in thickness could be readily cut in this way, and that this was thin enough for the purposes of this work. This method did not permit, however, any measurements being made on the bark other than its total thickness. Safranin and haematoxylin were used for staining in most cases, phloroglucin being used for some of the temporary mounts.

MEASUREMENT OF CAMBIAL ACTIVITY

Each section was measured by means of an ocular micrometer. Data were recorded for the thickness, measured along a radius, of the pith, one-year-old wood, new wood and bark. About fifteen sections were placed on each slide and were measured for each of the three pieces from each twig. An average for each slide was arrived at which was a mean of fifty to seventy-five measurements. The means of new wood measurements were then grouped and treated statistically, their mean with its probable error being ascertained (table 1). The differences between those of the check and those of the other series collected on the same day are given in table 2. The means of these classes are plotted, for the new wood, in figure 1. The data for the halves of trees are given in table 3.

TABLE 1

EFFECT OF DEFOLIATION ON RADIAL GROWTH IN NEW WOOD OF ONE-YEAR-OLD TWIGS OF McINTOSH APPLE IN 1922

Date	Check	Defoliated May 3	Defoliated June 6
	Microns	*Microns*	*Microns*
Apr. 19	....		
May 3	13.9 ± 2.5		
May 12	72.7 ± 3.9	66.0 ± 2.8	
May 24	266.3 ± 9.2	134.6 ± 6.3	
June 6	480.2 ± 15.7	116.6 ± 5.1	
June 20	582.5 ± 17.9	113.4 ± 5.5	475.6 ± 11.7
June 30	543.5 ± 14.4	140.1 ± 7.4	398.2 ± 11.7
July 11	927.0 ± 34.1	123.1 ± 2.1	492.5 ± 14.9
July 20	913.7 ± 29.9	130.0 ± 7.0	567.2 ± 12.8
Aug. 4	1100.6 ± 31.9		545.1 ± 12.8
Aug. 18	781.8 ± 25.6		
Sept. 8	819.9 ± 37.3	133.5 ± 5.2	519.7 ± 14.9

These data are shown graphically in figure 1.

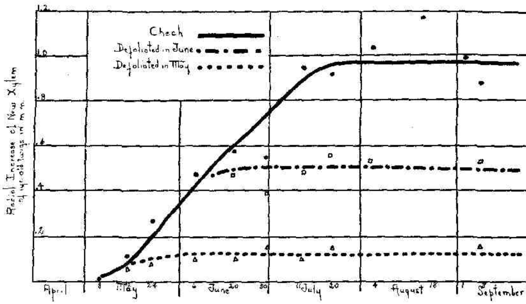

Fig. 1. The effect of defoliation on radial increase of new wood of one-year-old twigs of McIntosh apple.

TABLE 2

DIFFERENCES BETWEEN TREATED AND CHECK SERIES, AND BETWEEN TREATED SERIES

Date	Difference between check and defoliated May 3	Difference between check and defoliated June 6	Difference between defoliated May 3 and defoliated June 6
	Microns	*Microns*	*Microns*
May 12	6.7 ± 4.8		
May 24	131.7 ± 11.4		
June 6	363.6 ± 16.5		
June 20	469.1 ± 18.7	106.9 ± 18.7	362.2 ± 12.9
June 30	403.4 ± 16.2	145.3 ± 18.5	258.1 ± 13.4
July 11	803.9 ± 34.2	434.5 ± 37.2	369.4 ± 15.0
July 20	783.7 ± 30.7	346.5 ± 32.5	437.2 ± 14.6
Aug. 4		555.5 ± 34.3	
Sept. 8	686.4 ± 37.6	300.2 ± 40.1	386.2 ± 15.8

TABLE 3

MEASUREMENTS ON CHECK AND DEFOLIATED HALVES OF TREES OF MCINTOSH APPLE IN 1922—MEASUREMENTS OF NEW GROWTH OF ONE-YEAR-OLD TWIGS

Date	Check half	Defoliated half	Difference
	Microns	*Microns*	*Microns*
June 8*	460.4 ± 17.9	135.4 ± 9.6	325.0 ± 20.3
June 26†	630.9 ± 22.4	521.8 ± 33.5	109.1 ± 40.3
June 28*	944.8 ± 31.9	152.8 ± 12.8	792.0 ± 34.4
July 31*	1274.6 ± 68.3	148.5 ± 19.2	1126.1 ± 70.9
Aug. 10†	1237.6 ± 45.8	635.3 ± 25.6	602.3 ± 52.4

* Defoliated May 3. † Defoliated June 6.

Caliper measurements were made on two defoliated and two check trees. The data (table 4) show the same tendencies as those obtained by micrometer measurements up to the middle of July. After that there was an increase in the check, due probably to maturation and swelling of bark tissue.

TABLE 4

DIAMETER OF TWIGS AS SHOWN BY CALIPER MEASUREMENTS IN MILLIMETERS

Date	Check trees		Defoliated trees	
	2A	1C	3A	4A
May 3	4.83 ± .25	6.28 ± .47	5.46 ± .41	5.80 ± .21
June 6	5.73 ± .33	7.02 ± .49	5.66 ± .42	5.92 ± .23
June 26	6.20 ± .29	7.24 ± 52	5.68 ± .42	5.98 ± .23
July 20	6.30 ± .29	7.70 ± .59	5.66 ± .42	5.98 ± .23
Aug. 18	6.51 ± .33	8.02 ± .65	5.58 ± .40	5.96 ± .23
Sept. 8	6.80 ± .33	8.32 ± .59	5.64 ± .42	5.96 ± .23

The results recorded in tables 1, 2, 3, and 4 show clearly that growth is entirely checked within about two weeks as a result of defoliation. It is obvious that the growth that did occur was due to food from the stored reserve, since none was available from new leaves. Furthermore, the cessation of growth was not due to the death of the twigs, since they were able to continue to push out new leaves until the last of summer.

In order to be certain that the error due to unconscious selection of larger twigs in one series than in another was not a factor, the radii, exclusive of new wood, were compared. It was found that the average difference was 5±53 microns. This may be taken to show conclusively that differences in twig size are due to random sampling only, and play no significant part in the results reported.

A second set of trees in the same orchard was defoliated on May 4, 1923, as soon as the leaves unfolded. The methods were the same as those employed the year before. Collections were made on May 4 and 28 and on June 12. The data for this material are given in table 5.

TABLE 5

EFFECT OF DEFOLIATION ON THE RADIAL GROWTH OF NEW WOOD OF THE ONE-YEAR-OLD TWIGS OF McINTOSH, 1923

Date	Check	Defoliated	Difference
	Microns	*Microns*	*Microns*
May 4	0.0		
May 28	154.0 ± 5.80	96.0 ± 5.10	58.0 ± 7.70
June 12	347.0 ± 13.60	138.0 ± 6.10	209.0 ± 14.90

Another set of data was taken on different material. In this case one-year-old apple seedlings were grown in the greenhouse. This experiment was not highly successful because of the low percentage of trees that was brought out of the rest period at nearly enough the same date to be comparable. The trees were handled as follows:

November 11, 1922—Dug and heeled in out-of-doors.

January 30, 1923—Heeled in under greenhouse bench.

February 27, 1923—Potted in garden soil, ten trees to a seven-inch pot.

TABLE 6

RADIAL GROWTH OF NEW WOOD OF ONE-YEAR-OLD SEEDLINGS GROWN IN GREENHOUSE, 1923

Date	1 Check	2 Defoliated throughout	3 Defoliated from April 13 on	4 Defoliated to April 13 then allowed to grow	5 Same as Column 4 except defoliated again after May 15	6 Same as Column 3 except allowed to grow again after May 15	7 Buds removed as growth started
Apr. 25	134±18.3						
May 7	158±13.4	83±15.6					15±3.2
May 25	219±20.6				144±13.4	160±21.6	
May 30		115±12.4					63±7.8
June 7	198±21.2	111±10.0	111±16.3	266±21.1	134±11.5	169±19.3	

Of a total of nearly five hundred trees potted, about a third had to be discarded completely. Those remaining were divided into several series, as follows:

1. Check; no treatment.
2. Defoliated as in the experiments recorded above.
3. Defoliated after having grown normally until April 13.
4. Defoliated until April 13, and then allowed to grow normally.
5. The same as 4 until May 15, then defoliated again.
6. The same as 3 until May 15, then allowed to grow normally again.
7. All of the buds removed before they had unfolded.

Collections were made at irregular intervals and treated as in the earlier experiment. There was a much higher variability in this material than in the material taken from the orchard. This fact may perhaps be ascribed to genetic differences among the seedlings, as contrasted with the homogeneous constitution of the other population. This high variability rendered differences less obvious. Such differences as were found were, with a single exception, in the direction of the results recorded above. The data obtained from these seedlings are of little value by themselves, but tend to support the other data. The data from the sections of these trees are found in tables 6 and 7. The most striking result appears in series 7, that having the buds removed, where a large number of trees failed to survive the treatment. Of those that did survive few made more than a slight amount of growth.

TABLE 7

DIFFERENCES BETWEEN CHECK AND TREATED TREES

(Treatments numbered as in table 6)

Date	Treatment	*Difference from check in microns*
May 7	2	75 ± 20.5
May 7	7	143 ± 13.8
May 25	6	59 ± 29.8
May 25	5	75 ± 24.5
June 7	2	87 ± 23.4
June 7	3	87 ± 26.7
June 7	6	29 ± 28.6
June 7	4	+68 ± 29.9
June 7	5	64 ± 24.1

TABLE 8

TERMINAL GROWTH OF SEEDLINGS

Date	Series	Number of trees	Number of buds	Total growth *(Cm.)*	Growth per tree *(Cm.)*	Growth per bud *(Cm.)*
Mar. 28	1	49	247	271	5.5	1.09
Mar. 28	2	10	34	23	2.3	.67
Mar. 28	3	4	17	17	4.3	1.00
Mar. 28	4	12	71	37	3.1	.52
Mar. 28	6	17	77	71	4.2	.91
Mar. 28	5	20	95	72	3.6	.76
Apr. 13	1	61	292	601	9.8	2.13
Apr. 13	2	27	139	150	5.5	1.08
Apr. 13	3	5	24	14	3.8	.58
Apr. 13	4	16	104	308	19.2	2.96
Apr. 13	6	20	94	107	5.3	1.14
Apr. 13	5	29	152	385	13.3	2.53
May 7	1	15	99	303	20.2	3.06
May 7	2	8	55	130	16.2	2.36
May 15	1	37	144	560	15.1	3.88
May 15	2	32	137	309	9.7	2.25
May 25	6	10	71	268	26.8	3.77
May 25	5	10	92	240	24.0	2.61

In addition to those of radial increase, measurements were made of terminal growth on this material. Some of these measurements are given in tables 8 and 9.

TABLE 9

COMPARISON OF RADIAL AND TERMINAL GROWTH

Date	Series	Tree No.	Total terminal growth per tree (Cm.)	Number of buds	Growth per bud	Radial growth (Microns)
May 7	Check	I	10	4	2.5	166.0
		II	7	8	.88	141.1
		III	35	8	4.38	224.1
		IV	25	7	3.77	107.9
		V	15	1	15.00	141.1
		VI	30	12	2.50	74.6
		VII	30	15	2.00	149.4
		VIII	30	4	7.50	249.0
		IX	10	4	2.50	166.0
		X	20	6	3.33	166.0
		Average	21.2±2.16		4.44±.877	158.5±33.7
May 7	Defoliated	I	10	5	2.00	49.0
		II	12	2	6.00	33.2
		III	....	....	....	....
		IV	18	6	3.00	66.4
		V	20	12	1.67	99.6
		VI	28	18	1.55	24.9
		VII	25	5	5.00	58.1
		VIII	2	5	.40	24.9
		Average	16.4±2.29		2.80±.513	50.9± 6.8
		Difference	4.8±3.14		1.64±1,016	107.6±35.8

No definite correlation can be seen between radial and terminal growth, either total or per bud, nor was terminal growth governed so closely by the treatment. This lack of correlation may be due to the high variability of the material, however, and cannot be given too much weight. The fact that Harvey found a correlation between length growth and treatment tends to discount these results, since he worked within a clone, and should have had much more uniform material. The fact that terminal growth can continue after lateral growth has ceased indicates either a difference in ability to utilize such food as is present or a distribution of food in greater concentration to the terminal meristems.

The appearance of the wood formed after defoliation was much like that observed in the larch by Harper, viz., that it was thin-walled and resembled spring wood. This was especially noticeable in the series defoliated June 6, 1922. This type of wood was formed after summer wood formation had begun. There was no evidence that walls, thickened before defoliation, lost any material afterwards. Spring wood was also quite noticeable in series 6, which had two growth periods, where false annual rings were common.

A final set of material was collected from Red Astrachan apple trees located at the University Farm, Davis, California, in the spring of 1924. The period covered by this series was from March 7 to April 22, the critical period in the differentiation of the treated and untreated series. These trees were five years old. All treatments were given to halves of trees. Sufficient sections were made to determine that the response was the same as in the previous material sectioned.

These facts bring out clearly that cambial activity is dependent on the presence of leaves for its normal functioning. There is lacking the ability to function without the coöperation of the leaves.

The remainder of the work presented deals with the analysis of the material collected to determine whether or not any one of the groups: reducing sugars, non-reducing sugars, starch, hemi-celluloses or total nitrogen, can be designated as the limiting factor of growth under these conditions.

METHODS OF CHEMICAL ANALYSIS

Preparation of Material

The collections from the orchard made in 1922 were separated into wood and bark before further treatment was given. After finding that the curves for the two sets of analyses largely paralleled each other, it was decided to analyze the later series without this separation. This proved to be unwise, since the varying proportions of wood and bark introduced a very large error and the variability was found to be excessive. The material collected in California was divided into wood and bark. It is apparent, therefore, that the greater weight must be given the 1922 series and the 1924 series.

The wood and bark, and later the twigs as a whole, were dried at 78° to 80° C. This material was then ground to a coarse dust. A simple and very satisfactory device was used for grinding the wood. A large pencil sharpener was connected to a motor through a reduction gear. This gave a fairly uniform coarse powder that was fine enough to be readily extracted and coarse enough to be held by the alundum extraction thimbles. The bark was ground in a small coffee mill.

Analytical Procedure

For sugars, it was found that three hours' extraction in a Soxhlet extraction tube, with 95 per cent alcohol, gave complete removal. The time was determined by repeating the extraction with a second portion of alcohol, and determining the time beyond which no further power of reducing Fehling's solution was evident in the second extract. After extraction, the usual process of replacing the alcohol by distilled water, precipitation with neutral lead acetate, deleading with sodium sulphate, carbonate or oxalate, and estimation of reducing sugars by means of the Munson and Walker gravimetric method, was followed. It was found that filtering the solution through asbestos in a Gooch crucible gave such satisfactory elimination of organic materials that oxidation of the cuprous to cupric oxide gave no better results.

Total sugars were estimated by the same method, except that inversion of disaccharides was necessary. For this process the usual procedure of heating with normal hydrochloric acid to seventy degrees for fifteen minutes, followed by neutralization with sodium carbonate, was followed.

Each analysis is a composite of all twigs of each series collected on a given date. The average of duplicate determinations is given in each case except for those indicated, where a single sample was available. The variability in sampling was apparently insignificant. Starch extraction was found to be satisfactorily accomplished by a slight modification of the method of Sablon.[13] The material, after the sugars had been extracted, was freed from alcohol. The residue was wet with distilled water and autoclaved for two hours at fifteen pounds pressure. The time, two hours, was arrived at by experiment. Different lengths of time were tried, the other factors being the same. After extracting the products of digestion, the process was repeated. It was found that an hour and a half was about on the borderline between giving nothing and giving a slight amount above the blank. It seems necessary to partially disrupt the cells in order to allow the enzyme to come into contact with the starch. The material was cooled. Taka-diastase was added and the mixture allowed to stand overnight at room temperature. One hundred milligrams of taka-diastase were used in each case. At this point every sample was examined microchemically to see whether or not the starch had all disappeared. In a very few cases it was necessary to repeat the process before going farther.

The solution was separated from the residue and treated as for total sugars. The acid hydrolysis was deemed necessary because Davis and Daish[2] found dextrins to be present after digestion and to be removed to a certain extent by the process of precipitation.

Hemi-celluloses were estimated by digesting the residue from the starch extraction in normal hydrochloric acid under a reflux condenser for three hours. The acid was neutralized with sodium carbonate and the solution treated as for reducing sugars.

Total nitrogen was determined by means of the phenol-sulphuric modification of the Kjeldahl method.

RESULTS OF CHEMICAL ANALYSES

1. *Reducing Sugars.*

The results from the determination of reducing sugars, expressed as dextrose in per cent of dry weight, are given in table 10.

TABLE 10

EFFECT OF DEFOLIATION ON THE REDUCING SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	*Percent of dry weight as dextrose*					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
June 6	.49	1.66	.18	.84		
June 20	.60	2.61	.17	1.14	.47	1.65
July 11	.47*	2.16*	.22*	1.16*	.25*	1.04*
July 20	.41*	1.61*	.27*	1.65*	.45*	1.66*
Aug. 4	.26	1.66			.29	1.48
Aug. 18	.30	1.71				
Sept. 1	.24	1.27				
Sept. 8	.26	1.47	.38*	1.79*	.44*	1.83*

* Single determination.

The same data are shown graphically in figure 2. The data for half trees collected June 8 are: check, wood .74 per cent, bark 2.20 per cent; defoliated, wood .46 per cent, bark 1.24 per cent. These cover only 1922 material and only a portion of that because of the lack of material. The points indicated by these analyses are:

1. The concentration of reducing sugars is very much greater in the bark than in the wood.

2. At the period covered by these analyses, i.e., from more than a month after growth started until the end of the season, the concentration of reducing sugars has been somewhat reduced in the defoliated series, as compared with the check. It is unfortunate that the material for those collections made immediately after defoliation

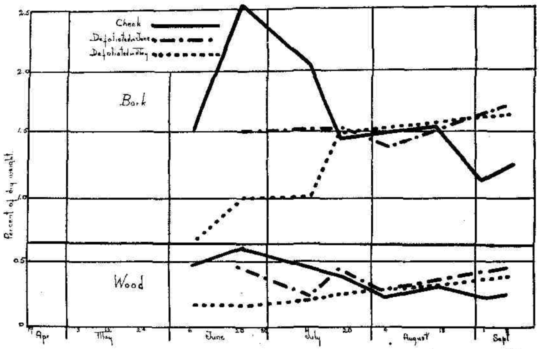

Fig. 2. Effect of defoliation on reducing sugar content of one-year-old twigs of McIntosh apple.

was exhausted before determinations for reducing sugar were made. The fact that some time had elapsed after defoliation makes the significance doubtful. However, the California material indicates that the reduction in amount takes place very soon after defoliation.

3. The reduction in reducing sugars that is noticed does not appear to be sufficient to account for the cessation of cambial activity, which occurred in every case of the 1922 series before the analyses were made. It would seem that the concentrations indicated are adequate for growth, from the data that are available on this point.

4. Considered with the data for total sugars and starch presented below, there is the suggestion that enzyme activity has been retarded. A partial inactivation of enzymes might account for the phenomena noted.

5. There are fluctuations of considerable magnitude between different collections. These seem to be readily accounted for only by the assumption of differences of this magnitude between different trees.

2. *Total Sugars.*

The data for total sugars are somewhat more extensive than are those for reducing sugars. The results of the analyses for the same material are given to table 11.

TABLE 11

EFFECT OF DEFOLIATION ON THE TOTAL SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent of dry weight as dextrose					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19	.68	3.35				
May 3	.59	1.86				
May 12	.79	6.15	1.18*	6.05		
May 24	.95	4.14	.77	4.34		
June 6	1.00	3.98	.65	3.42		
June 20	.73	3.63	.45	2.55*	.79	2.49
June 30	.65	3.03	.43	1.76	.84	1.95
July 11	.67	2.84	.37	1.87	.42	1.76
July 20	.40	2.54	.34	2.13	.44	2.67
Aug. 4	.35	1.95			.38	1.04
Aug. 18	.38	1.97				
Sept. 1	.27	2.21				
Sept. 8	.35	2.09	.58*	2.58*	.60*	2.34*

* Single determination.

The data are shown graphically in figure 3. The results, as in the preceding case, are based on the percentage of dry weight, expressed as dextrose. For the June 8 collection of halves of trees, the percentages are: Check, wood 1.25, bark 6.46; defoliated, wood 1.91, bark 4.22. The results indicate about the same things as do reducing sugars. There is the same marked difference in concentration between wood and bark. There is a better basis of comparison between check and treated series through a more nearly unbroken series of determinations. The differences are not so marked as in the case of reducing sugars. At the beginning of the season, when growth was stopping in the first series defoliated, there was as much total sugar in one as in the other. Fluctuations are less also between collections. There is thus an even smaller suggestion than in the case of reducing sugars that the sugar fraction, at least as a group, is the limiting factor under the conditions of this experiment.

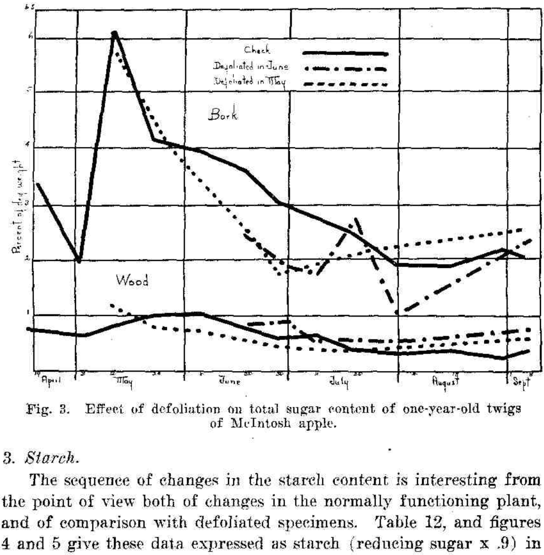

Fig. 3. Effect of defoliation on total sugar content of one-year-old twigs of McIntosh apple.

3. *Starch.*

The sequence of changes in the starch content is interesting from the point of view both of changes in the normally functioning plant, and of comparison with defoliated specimens. Table 12, and figures 4 and 5 give these data expressed as starch (reducing sugar x .9) in per cent dry weight. For the June 8 collection from halves of trees, the percentages are: Check, wood 5.94, bark 10.11; defoliated, wood 7.26, bark 11.93. The normal sequence is similar in most respects to the curves published by numerous workers in the past. There is the usual high initial concentration followed first by a drop almost to zero and then by a gradual rise throughout the remainder of the season. The most striking thing about the analyses is the enormous fluctuation in the bark between successive collections. There is apparently a very high starch deposition in the cortical region of the bark, forming a considerable share of the reserve. As was noted in the case of reducing sugar, there is a suggestion of inactivation of diastase.

The starch disappears more slowly from the defoliated series than from the check. This gives additional evidence, however, that it is not a lack of carbohydrates that is the limiting factor in the checking of growth.

TABLE 12

EFFECT OF DEFOLIATION ON THE STARCH CONTENT OF ONE-YEAR-OLD TWIGS OF THE MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight as dextrose x .9					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19	4.87	6.95				
May 3	9.28	5.53				
May 12	1.16	6.34	3.21	5.68		
May 24	1.58	2.11	2.39	2.38		
June 6	2.43	5.60	2.94	5.36		
June 20	2.92	2.97	1.48	2.27	1.35	1.60
June 30	3.97	6.11	2.45	6.35	2.48	4.63
July 11	3.06	3.74	1.80	2.10	1.71	1.98
July 20	6.26	5.38	1.31	2.78	1.50	2.53
Aug. 4	3.06	6.20			1.60	2.16
Aug. 18	4.62	9.07				
Sept. 1	6.15	10.23				
Sept. 8	5.54	10.24	2.84	5.01	2.00	3.69

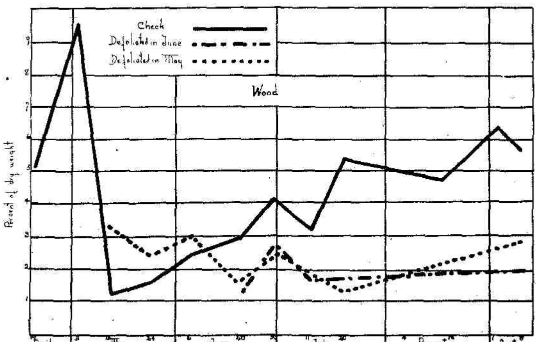

Fig. 4. Effect of defoliation on starch content of the wood of one-year-old twigs of McIntosh apple.

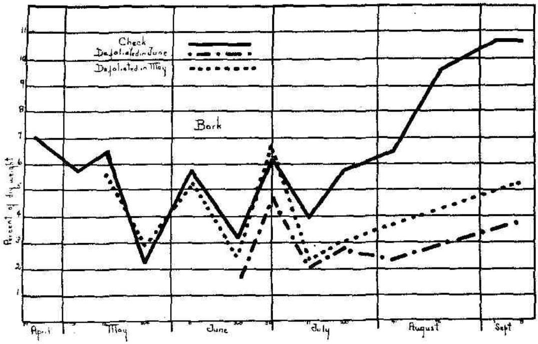

Fig. 5. Effect of defoliation on starch content of the bark of one-year-old twigs of McIntosh apple.

4. *Hemi-Celluloses.*

The analyses for hemi-cellulose (table 13 and fig. 6) show nothing. The half tree analyses are again in accord, June 8 halves given: Check, wood 22.98, bark 10.20; defoliated, wood 24.33, bark 10.69. The data again are expressed as dextrose x .9 in per cent dry weight. There are fluctuations, but they seem to be slight and of no significance. This series was early discontinued. If hemi-cellulose can be utilized as a reserve food, it seems not to have been in the case of

TABLE 13

EFFECT OF DEFOLIATION ON THE HEMI-CELLULOSE CONTENT OF ONE-YEAR-OLD TWIGS OF McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight as dextrose x .9					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	19.10	12.81				
May 12	20.24	12.14	20.18	11.55		
May 24	20.88	13.87	21.46	12.37		
June 6	18.47	11.52	20.03	13.00		
June 20	19.02	11.42	17.98	11.32	18.81	12.14
June 30	19.30		20.45			
July 11	19.41	12.06	19.79	11.83	18.12	12.28

this material. There certainly is nothing here to indicate carbohydrate deficiency. A point of interest, however, is the low content in the bark as compared to that of the wood.

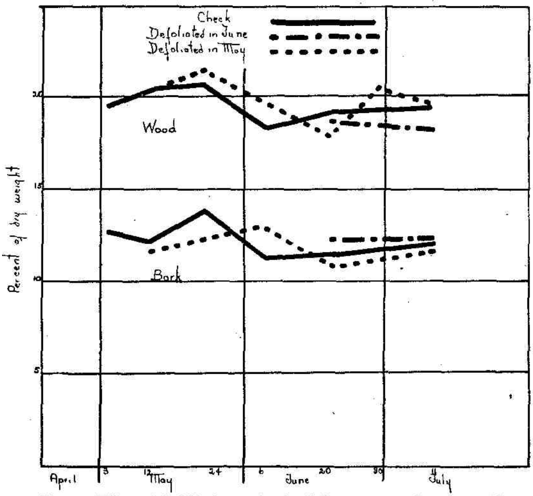

Fig. 6. Effect of defoliation on hemi-cellulose content of one-year-old twigs of McIntosh apple.

5. *Total Nitrogen.*

Finally, there are the analyses for total nitrogen expressed in per cent dry weight (table 14 and fig. 7). A large difference in the nitrogen content between the wood and bark is shown, in the same direction as in the case of the sugars. The total nitrogen content of the check series falls off steadily throughout the summer. The defoliated series, however, shows no such falling off. In the bark there is a gradual increase, and in the wood a slight decrease. The series defoliated in June, while not showing the rise seen in the first series, does in the bark show a distinct check in the rate at which it falls off and maintains a generally higher level than does the check. The wood, with a much smaller content, is not so regular. There seems to be much less fluctuation between trees than is the case with the carbohydrates. There is no suggestion whatever that total nitrogen is a limiting factor.

TABLE 14

EFFECT OF DEFOLIATION ON THE TOTAL NITROGEN CONTENT OF ONE-YEAR-OLD TWIGS OF THE McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19		1.19*				
May 3	.51*	1.24				
May 12	.53	1.00	.59*	1.08		
May 24	.51	.91	.69	1.19		
June 6	.34	.92	.40	1.17		
June 20	.43	.73	.61	1.29	.38	1.18
June 30	.40*	.88	......	1.61	......	1.04
July 11	.42	1.02	.48	1.37	.42	1.13
July 20	.14	.59	.33	1.16	.23	.84
Aug. 4	.10	.62			.13*	.83*
Aug. 18	.18	.60				
Sept. 1	.18	.56				
Sept. 8	.27	.67	.42	1.23	.20	.82
For halves of trees						
June 8	.30	.80	.36	1.04		
June 28	.20	.67	.30	.90		
June 26	.21	.67			.23	.68
Sept. 10	.14	.62			.16*	.67*

* Single determination.

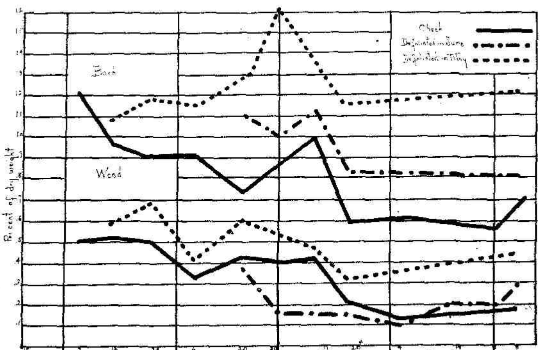

Fig. 7. Effect of defoliation on total nitrogen content of one-year-old twigs of McIntosh apple.

6. *Carbohydrate-Nitrogen Ratio.*

The only thing remaining to be done with these data, then, is to determine whether or not the carbohydrate-nitrogen ratio, or rather the starch-nitrogen or the sugar-nitrogen ratio, can be presented as a cause for the phenomenon. Table 15 shows the starch-nitrogen ratio for the collections up to and including July 20. Generally there is a higher ratio in the wood than in the bark. This is, of course, what would be expected. The ratio is uniformly lower in the bark of the defoliated trees than in that of the check.

TABLE 15

EFFECT OF DEFOLIATION ON STARCH-NITROGEN RATIOS IN ONE-YEAR-OLD TWIGS OF MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	18.2	4.4				
May 12	2.2	6.4	5.4	5.3		
May 24	3.1	2.3	3.5	2.0		
June 6	7.1	6.1	7.3	4.6		
June 20	6.7	4.0	2.4	1.8	3.6	1.4
June 30	9.9	7.0		3.9		4.4
July 11	7.4	3.7	3.8	1.5	4.1	1.7
Halves of trees						
June 8	19.5	12.5	20.0	11.5		

The ratio of total sugar to nitrogen (shown in table 16) was calculated for the same material used for table 15. The ratios found for wood are again inconclusive. Those for bark are more consistent than were those in table 15, and all deviate in the same direction as do those in table 15, that is, the sugar-nitrogen ratio is less in the bark of defoliated series than in the corresponding checks.

As has alrealy been indicated, the analyses of whole twigs are given little weight in this discussion. Therefore, the writer will be content to present the data in table 17 for the McIntosh orchard for 1923 and table 18 for the greenhouse-grown seedlings with little comment. The former seem to show a slightly greater reduction in carbohydrates than was found in the previous season's analyses, but in general substantiate the earlier results. As was to be expected, the variability in composition of the seedlings is very much greater than in the other series. There are no seriously conflicting data. The series having the buds removed gave the most striking results, as it did in the growth measurements. It indicates, perhaps, that the difference in carbohydrates is more to be associated with the amount of material used in pushing out new length growth than with cambial activity. This would account for the fact that this series shows such a slight reduction in carbohydrates as compared with those series which were allowed to form terminal growing points.

TABLE 16

EFFECT OF DEFOLIATION ON THE SUGAR-NITROGEN RATIOS IN ONE-YEAR-OLD TWIGS OF THE McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	1.1	1.1				
May 12	1.5	6.1	2.0	5.7		
May 24	1.9	4.6	1.1	3.6		
June 6	3.0	4.3	1.6	2.9		
June 20	1.7	4.9	.7	2.0	2.1	2.2
June 30	1.6	3.5		1.1		1.9
July 11	1.6	3.8	.8	1.4	1.0	1.5
Halves of trees						
June 8	4.2	8.1	2.5	4.0		

TABLE 17

EFFECT OF DEFOLIATION ON THE CHEMICAL COMPOSITION OF ONE-YEAR-OLD TWIGS OF THE McINTOSH APPLE, 1923. WHOLE TREES TREATED. WHOLE TWIGS ANALYZED

Date	Reducing sugars % dry weight		Total sugars % dry weight		Starch % dry weight		Total nitrogen % dry weight	
	Check	Defoliated	Check	Defoliated	Check	Defoliated	Check	Defoliated
May 4	1.81		1.91		4.44*		.73*	
May 28	1.28	.99*	1.18	.94*	.76	.72	.52*	.61*
June 12	1.60	.79	1.78*	.89	1.35	.93	.52	.63

* Single determinations.

TABLE 18

EFFECT OF DEFOLIATION AND DISBUDDING ON THE CHEMICAL CONTENT OF ONE-YEAR-OLD SEEDLINGS, 1923. WHOLE TWIGS USED FOR ANALYSIS. PER CENT DRY WEIGHT

Date	Treatment*	Reducing sugar % dry weight	Total sugar % dry weight	Starch % dry weight	Total nitrogen % dry weight
May 7	1	.79	.95	1.85	.56
	2	.48†	.63†	.87	.32†
	7	.68†		2.83†	.70†
May 25	1	.99	1.11	2.42	.55
	5	.52		.83	.53
	6		1.18	.60	.50
May 30	2				.51†
	7				.77†
June 7	1	.73	1.05	3.04	.54
	2	.35†		1.30	.67
	3	.55†	1.18†	.74	.65†
	4				.45†
	5				.52†
	6				.59†

* Treatment—1—Check.
—2—Defoliated throughout.
—3—Defoliated after April 13.
—4—Defoliated until April 13, then allowed to grow.
—5—The same as 4 until May 15, then defoliated again.
—6—The same as 3 until May 15, then allowed to grow again.
—7—Disbudded.

† Single determinations.

TABLE 19

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE REDUCING SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF RED ASTRACHAN APPLE, 1924. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	.98	2.46							
Mar. 15	.94	1.81							
Mar. 20	1.01	2.30	1.00	1.69	Mar. 18	.99	1.49	.79	1.59
Mar. 25	.81	2.05	.57	1.58	Mar. 25	1.02	1.68	.69	1.75
Apr. 8	1.54	2.30	1.24	2.04					
Apr. 22	1.13	2.02	.87	2.45					

TABLE 20

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE TOTAL SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF RED ASTRACHAN APPLE, 1924. PER CENT DRY WEIGHT. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	1.07	2.72							
Mar. 15	1.18	3.71							
Mar. 20		3.15		2.63	Mar. 18	1.08	4.45	.94	4.18
Mar. 25	.97	4.89	.68	4.59	Mar. 25	1.51	4.77	1.24	4.62
Apr. 8	2.41	5.67	2.18	6.54					
Apr. 22	1.83	5.89	1.35	5.26					

TABLE 21

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE STARCH CONTENT OF ONE-YEAR-OLD TWIGS OF RED ASTRACHAN APPLE, 1924. HALF TREES TREATED. PER CENT DRY WEIGHT

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	2.84	3.20							
Mar. 15	2.62	2.52							
Mar. 20	2.48	2.56	3.09	3.40	Mar. 18	2.26	2.54	2.39	2.42
Mar. 25	2.06	1.52	1.93	1.90	Mar. 25	2.12	1.26	2.15	1.62
Apr. 8	1.11	1.02	1.13	1.25					
Apr. 22	0.00	.77	0.00	.77					

TABLE 22

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE TOTAL NITROGEN CONTENT OF ONE-YEAR-OLD TWIGS OF RED ASTRACHAN APPLE, 1924. PER CENT DRY WEIGHT. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	.50	.94							
Mar. 15	.51	.95							
Mar. 20	.48	.94	.50	.98	Mar. 18	.45	.91	.49	.92
Mar. 25	.47	.80	.50	.88	Mar. 25	.43	.87	.42	.93
Apr. 8	.35	.74	.45	.80					
Apr. 22	.48	.74	.43	.78					

DISCUSSION AND CONCLUSIONS

The actual factor limiting growth under conditions of defoliation has been a matter of speculation by a number of writers. Harper[4] has summarized the theories heretofore brought forward as follows:

1. Wieler and Hartig suggest lack of food.

2. Schwarz, lack of pressure from bending movements.

3 Strasburger and Haberlandt, the need of the plant for an increased water supply.

4. Lutz, an excess of water in the tissues due to decreased transpiration. Harper himself favors the first idea, though he presents nothing to substantiate the suggestion.

Harvey[5] has added another in the form of the carbohydrate-nitrogen ratio.

Roberts[12] suggested lack of nitrogen, a phase of the first.

Considering these suggestions, it seems from the data presented by Harvey and by the writer that the lack of total nitrogen is not the factor sought under these conditions. The fact that Roberts' work was done at a different time of year, and with different material may explain the discrepancy.

The starch-nitrogen ratios calculated for this material do not in the writer's estimation, adequately account for the observed results. The ratio is subject to such wide fluctuations and the differences between the series are generally so slight that to assign this as the controlling factor in limiting growth seems to be an unwarranted assumption. Although sugar-nitrogen is less variable, at least in the bark, the differences do not appear to be so great at first as later, and this ratio also seems not to be the limiting factor, though a definite conclusion cannot well be drawn. The possibility still remains that it is not total nitrogen, but some fraction of it which may be the essential thing in the ratio, but there are insufficient data on this point to warrant a discussion of the idea. The fact that Nightingale[9] has found it necessary to modify Kraus's original postulates along this line may perhaps lend color to the suggestion.

Harvey's data on the moisture content of the different series may seem to furnish a basis for Lutz' proposal. It seems that larger differences in water content would be necessary to effect such radical changes in the metabolism of the plant as those observed, especially in view of the fact that differences between the bases and tips of a series of a given treatment were generally greater than between series of different treatments. Perhaps these differences can be largely attributed to differing proportions of wood and bark, since the proportion of wood is much greater in the base than in the tip. That water pressure may delay growth has been pointed out by Heinicke,[6] though the differences in moisture content that accompanied this phenomenon are not given. Moisture content is of doubtful significance at best, and it is questionable whether this suggestion can be given much weight.

The least acceptable theory given above is that of "the need of the tree for an increased water supply." The data available indicate an excess of water rather than a deficiency, while at the same time the "need" for it is reduced by the lack of leaf surface. These considerations, in addition to the natural reaction against a teleological explanation, dispose of this idea.

Schwarz' hypothesis does not seem to have been very well received by recent investigators. It may be said to have received some support from the distribution of xylem in the annual rings of trees growing in a region of strong winds that are uniformly from one direction. However, the fact that plants develop without this stimulus under greenhouse conditions indicates that it is probably a minor factor.

Neither the analyses of Harvey nor those here given are of much value in supporting the idea of starvation. It still remains a possibility that starvation, due to the lack of some specific compound, either a fraction of one of the groups treated or some unrelated compound, may be the true explanation of the condition.

A closely related idea is that of growth promoting substances. It has been suggested that many activities of the plant are regulated by hormones. If these are produced by the leaves, the supply might be cut off by defoliation. This idea is highly speculative, however.

The hypothesis that the presence of inhibitory substances, perhaps of the nature of the "staling" substance of fungous cultures, may be responsible, is also speculative. It might be assumed that the leaves are effective in removing or counteracting the effect of such substances.

A more promising hypothesis, and one suggested by the data, is the partial or complete inactivation of enzyme activity. It has already been suggested that such an assumption is in accord with the relations observed between the various carbohydrates. This assumed inactivation might be brought about by a change in the hydrogen-ion concentration of the cell sap, by the failure of the zymogen to change to the active enzyme, or by the formation of compounds between the enzyme and other substances in the cell, perhaps the substrate, with subsequent failure to disjoin. A change in hydrogen-ion concentration, again, might have other effects than that indicated above. The most obvious is a change in the proteins, as indicated by the work of Loeb, either by precipitation of the protein at the iso-electric point, or the change from one type of compound to another. The strong buffer action of cell sap does not support this idea.

The localization of certain compounds in certain tissues, as indicated by the difference in wood and bark, may be carried much farther. That is, the meristematic region may be suffering from a lack of certain foods while a closely adjacent tissue may have an abundance of the substance, and through the failure of lateral translocation, be unable to supply it to the point where it is needed for growth. This is further suggested by the fact that terminal meristems are able to continue growth long after the lateral one has ceased to function. The analysis of a group of tissues such as those present in the bark would effectually mask the relations between them.

The possibilities outlined above indicate that there is nothing that can be legitimately concluded as to the relation of foods to the activity of the cambium except that totals of the groups above indicated are not the limiting factors. The indications point away from starvation, but do not eliminate it. The most plausible hypotheses appear to the writer to be:

1. Enzyme inactivation, with consequent lack of certain end products of digestion necessary for growth.
2. Starvation, due to failure of the leaves to supply certain compounds directly, or to failure of translocation through short distances.
3. Lack of balance of carbohydrate-nitrogen ratio, calculated not on totals but on fractions that may prove to be more directly involved.

SUMMARY

1. Defoliated apple trees, or halves of trees, showed a cessation of radial increase of wood within two weeks after defoliation.
2. This was accompanied by a modification of the thickness of the walls of the cells laid down after defoliation.
3. This phenomenon does not seem to be associated with a deficiency of stored food as indicated by analyses for reducing sugar, total sugar, starch, hemicellulose, and total nitrogen in the wood and in the bark.
4. There are several alternate theories that might be suggested to account for the phenomena.

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