A Preliminary Report on the Effects of Summer Pinching upon the Carbohydrate and Nitrogen Contents of the Japanese Pear Shoots * *

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In Japan, the fruit growers pinch not rarely in summer the vigorously growing shoots of fruit trees, intending to get flower buds on them, and it is believed that this practice brings about in the pinched shoots the carbohydrate accumulation, and the carbohydrate-nitrogen ratio becomes, consequently, high enough to differentiate flower buds, for the growing tip of shoots, which spends carbohydrates much, is removed and the elongation of shoots is checked by pinching. It is, however, doubtful whether the carbohydrate accumulation is actually produced by pinching, and no investigation which studied the chemical changes taken place in the pinched shoots, has been reported.

The present brief report of the investigation which is one phase of the studies on the physiological foundation of the tree responses to pruning, deals with the changes in chemical constituents of the pinched shoots of the Japanese pear trees.

Material and Methods

Three, circ. 20-year-old Japanese pear trees (*Pyrus serotina* Rehder) of the variety Imamuraaki, grown in the orchard of the Tokyo Imperial University at Komaba, Tokyo, were selected for the study. Many pairs of shoots were chosen for uniformity, and tabulated, one of each pair being pinched and the other remaining untreated as check. In the pinching treatment of this study, the soft terminal portion of shoots was removed at the point 2 or 3 cm. below the tip with the nails of fingers. After several weeks the pinched and the untreated shoots were collected, immediately stripped of leaves and the fresh weights determined.

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Methods of Chemical Analysis

Preservation. The samples were clipped as rapidly as possible into small pieces from one to three mm. long by means of a pruning shears. The pieces were tossed into an Erlenmeyer flask, and after adding calcium carbonate and enough 95 per cent alcohol so as to make the final concentration 80 per cent, the flask was heated on the water bath to gentle boiling for 30 minutes.

Extraction. The alcohol was poured off of the residue and filtered through a hardened paper. The residue was dried in a large evaporating dish and ground to pass through an 80-mesh sieve and extracted with 80 per cent alcohol using an Allin's reverse condenser until the percolates were colorless.

Analysis of the extract. (1) Nitrogen. The extract was cleared with neutral lead acetate and the diphenylamine nitrate test was performed. In no case a positive test being obtained, determinations were made by the Kjeldahl method. The distillation was made with the Shioiri-Okuda's apparatus using methyl red indicator with 0.02N sodium hydroxide. (2) Carbohydrates. The aliquots of extract which had been evaporated were taken up in water, cleared with neutral lead acetate and decalcified with sodium oxalate. Reducing power was determined by Fehling's solution. The cuprous oxide was determined by the Bertland method. Non-reducing sugars and glucoside were hydrolysed using dilute hydrochloric acid.

Analysis of the residue. (1) Carbohydrates. a, *Water soluble polysaccharides.* A 2gm. sample of the residue was extracted with water for 12 hours. The results were calculated as dextrose. b, *Starch.* The residue from the above treatment was washed into an Erlenmeyer flask and heated in the boiling water bath for 30 minutes to gelatinize starch. After cooling to 38°C., an aliquot portion of a solution of taka-diastase was added so that 0.1 gm. of enzyme preparation was present. It was then incubated for 48 hours with 2cc. of toluene. At the end of this period the solution was heated to boiling in the boiling water bath for 15 minutes to render the enzymes inactive. The results were calculated by the R. L. Shriner method. c, *Acid-hydrolysable polysaccharides.* The insoluble residue from the starch determination was transferred to a flask. After adding water and dilute hydrochloric acid the flask was heated under air condenser for 2½ hours in a boiling water bath. The results were calculated as dextrose. (2) Nitrogen. Nitrogen was determined by the Kjeldahl method as mentioned in the case of soluble nitrogen.

Results

In 1931, the shoots were pinched on June 16, and the samples for chemical analysis were taken every seven days after the treatment.

A summary of the chemical analysis of the untreated and the pinched shoots is presented in Table 1. In this table, we cannot see any difference in the carbohydrate and nitrogen contents between the untreated and the pinched shoots. The seasonal changes of the carbohydrate and nitrogen contents in the Japanese pear shoots were in agreement with those in the apple shoots reported by Harvey (1). Namely, water, soluble solids, and soluble and insoluble nitrogen decreased through the growing season, and insoluble solids, sugars, polysaccharides, total carbohydrates, and the carbohydrate-nitrogen ratio increased as the season progressed.

In 1931, the shoots were pinched when their most active growth nearly ended (Fig. 1), and it was thought that pinching might be conducted too late to bring about

Table 1.— Seasonal Changes of the Chemical Composition in the Untreated and the Pinched Shoots of the Japanese Pear Trees-1931, Results expressed upon the Dry Weight Basis, The Shoots pinched on June 16.

	Untreated						
	June 30	July 7	July 14	July 21	July 28	Aug. 4	Aug. 11
Water	261	216	185	207	181	177	167
Soluble solids	20.01	17.79	16.09	14.59	12.97	12.17	7.78
Insoluble solids	79.99	82.21	83.91	85.41	87.03	87.83	92.22
Direct reducing sugars	3.06	2.94	3.61	2.33	2.92	3.41	4.59
Non-reducing sugars	3.82	3.52	4.83	3.24	3.48	4.02	5.29
Water-soluble polysaccharides	0.38	0.40	0.32	0.52	0.39	0.63	0.50
Starch	1.14	1.38	1.29	1.39	1.37	1.56	1.69
Acid-hydrolysable polysaccharides	4.68	5.17	4.54	4.68	6.30	6.37	7.93
Total carbohydrates	13.08	13.41	14.57	12.14	14.46	15.99	20.00
Soluble nitrogen	0.29	0.24	0.22	0.20	0.18	0.16	0.15
Insoluble nitrogen	0.86	0.81	0.81	0.77	0.77	0.77	0.78
Total nitrogen	1.05	1.05	1.03	0.97	0.95	0.93	0.93
C/N	12.46	12.77	14.15	12.52	15.22	17.19	21.51

	Pinched						
	June 30	July 7	July 14	July 21	July 28	Aug. 4	Aug. 11
Water	272	221	178	196	188	177	158
Soluble solids	16.13	17.07	15.44	14.52	13.60	10.29	8.94
Insoluble solids	82.87	82.93	84.56	85.48	86.40	89.71	91.06
Direct reducing sugars	2.63	3.07	3.47	2.26	3.64	3.48	4.11
Non-reducing sugars	3.27	3.64	3.98	3.01	4.12	3.89	5.34
Water-soluble polysaccharides	0.39	0.41	0.43	0.57	0.40	0.65	0.50
Starch	1.13	1.18	1.29	1.36	1.40	1.54	1.60
Acid-hydrolysable polysaccharides	4.66	5.55	4.60	4.61	6.27	6.50	7.22
Total carbohydrates	12.08	13.85	13.77	11.81	15.83	16.06	18.77
Soluble nitrogen	0.23	0.23	0.18	0.18	0.17	0.17	0.16
Insoluble nitrogen	0.90	0.88	0.83	0.81	0.81	0.83	0.78
Total nitrogen	1.13	1.11	1.01	0.99	0.98	1.00	0.94
C/N	10.69	12.48	10.73	11.93	16.15	16.06	19.97

Fig. 1. Growth Curve of the Imamuraaki Pear Shoots

remarkable changes in chemical constituents. Moreover, a collected shoot was treated in chemical analysis as a whole, not being separated into terminal and basal portion.

In 1932, therefore, the shoots were pinched on May 15, when they were making rapid growth, and collected together with check shoots on July 5. When the shoots were pinched, their average length was 47.32 cm., and when the shoots were collected, the untreated shoots were 97.32 cm. long in average. The second growths

produced on the pinched shoots were removed soon after they appeared. The collected untreated shoots were divided into four portions: (1) the tip, which consisted of the upper 3 to 4 cm.; (2) the upper middle, which consisted of 25 to 30 cm. upper part next to the tip; (3) the lower middle, which consisted of 25 to 32 cm. part above the base and the upper end of this portion corresponded to the point at which the other shoot of its pair was pinched; (4) the base, the lower 6 to 8 cm. The pinched shoots were divided into two halves, upper and basal.

The results of the chemical analysis in 1932 are summarized in Tables 2 and 3. Comparing the carbohydrate and nitrogen contents of the pinched shoots with those of the base and the lower middle portion corresponded to the pinched shoots in the untreated shoots, the percentages of reducing sugars, water soluble polysaccharides and starch were very slightly larger in the pinched shoots, but that of acid-hydrolysable polysaccharides was smaller, and the amounts of total carbohydrates were slightly smaller in the pinched shoots than in the corresponded portions of the untreated shoots. The

Table 2.— Chemical Constituents of the Untreated and the Pinched Shoots,-
1932. Per Cent Dry Weight. The shoots pinched on May 15, the
Samples collected on July 5.

	Untreated				Pinched	
	Base	Lower middle	Upper middle	Tip	Basal half	Upper half
Water	125	150	170	208	131	146
Soluble solids	14.87	16.52	16.65	24.91	14.28	15.41
Insoluble solids	85.13	83.48	83.35	75.09	85.72	84.59
Direct reducing sugars	2.77	2.29	2.24	2.11	2.82	2.91
Non-reducing sugars	2.63	3.75	3.65	2.61	3.03	2.64
Water-soluble polysaccharides	0.69	0.69	0.68	0.54	0.74	0.73
Starch	0.93	0.89	0.79	0.65	1.02	0.97
Acid-hydrolysable polysaccharides	6.45	5.26	4.94	3.91	5.78	5.12
Total carbohydrates	13.47	12.88	12.30	9.82	13.39	12.37
Soluble nitrogen	0.13	0.13	0.18	0.53	0.13	0.36
Insoluble nitrogen	0.58	0.64	0.75	1.91	0.72	1.15
Total nitrogen	0.71	0.77	0.93	2.44	0.85	1.51
C/N	18.97	16.73	13.23	4.02	15.75	8.19

Table 3.— Chemical Constituents of the Untreated and the Pinched Shoots,-
1932. Per Cent Fresh Weight.

	Untreated				Pinched	
	Base	Lower middle	Upper middle	Tip	Basal half	Upper half
Water	55.61	60.06	62.90	67.56	56.59	59.36
Soluble solids	6.60	6.60	6.72	8.08	6.20	6.28
Insoluble solids	37.79	33.34	30.38	24.36	37.21	34.36
Direct reducing sugars	1.25	0.92	0.90	0.68	1.22	1.18
Non-reducing sugars	1.19	1.50	1.47	0.85	1.31	1.07
Water-soluble polysaccharides	0.33	0.33	0.30	0.23	0.37	0.35
Starch	0.52	0.43	0.35	0.23	0.52	0.47
Acid-hydrolysable polysaccharides	3.09	2.42	2.21	1.69	2.93	2.46
Total carbohydrates	6.38	5.60	5.23	3.73	6.35	6.03
Soluble nitrogen	0.05	0.05	0.04	0.17	0.06	0.14
Insoluble nitrogen	0.31	0.31	0.34	0.83	0.36	0.55
Total nitrogen	0.36	0.36	0.38	1.00	0.42	0.69
C/N	17.72	15.56	13.89	3.73	15.12	8.74

pinched shoots, especially its upper portions, were evidently richer in nitrogen contents, both soluble and insoluble nitrogen, than the corresponded portions of the cheek shoots.

From the data presented it will be said that pinching increases the percentage of nitrogen, while it affects very little the carbohydrate contents, and the carbohydrate-nitrogen ratio becomes consequently lower in the pinched shoots, just in opposition to the believed conception.

In Tables 1 and 2, it can be also noticed that the regional changes in chemical constituents of the Japanese pear shoots are in accord with those of the apple shoots reported by Harvey (1). Those substances, water, soluble solids, and nitrogen, which decreased through the growing season, were most abundant in the tip of the shoots and least abundant in the base, and conversely, those substances, insoluble solids, sugars, polysaccharides, total carbohydrates, and the carbohydrate-nitrogen ratio, which increased through the growing season, were least abundant in the tip and most abundant in the base.

Discussion

The chemical changes produced in the ringed plus defoliated shoots in Harvey's experiments with apples (1) are very interest in relation to the present investigation. In his ringing plus defoliation treatment the shoots were ringed at the point 6 to 9 cm. above the base when they grew to be 30 to 40 cm. long and the leaves on the portion above the ringed point were removed. The most remarkable effect produced in the basal portion of the ringed plus defoliated shoots was rather large increase in percentages of nitrogen, and the total carbohydrates varied in the opposite direction but the changes were relatively not so great. From this result obtained by Harvey it may be suggested that the summer pruning will increase nitrogen greatly and decrease carbohydrates, because, as Harvey noticed, this treatment would give nearly the same effects on the chemical composition of the basal portion below the ringed point with those produced by a complete removal of the upper portion of the shoots, i. e., summer pruning.

In his pruning studies with apples Hooker (2) reported that winter pruning (heading) induced a rapid increase in the amount of nitrogen and decreased that of starch and sugar in the cut ends of the shoots.

Pruning, either summer or winter pruning, is, therefore, a treatment which causes a fall in the value of the carbohydrate-nitrogen ratio, increasing nitrogen and decreasing carbohydrates. As pinching practised in the present experiment is a kind of summer pruning, it may be reasonable that pinching produced the chemical changes same in trend with those which should be induced by pruning.

If pinching produces carbohydrate accumulation on account of removal of the growing point which spends much carbohydrates, then in the untreated normal growing shoots the rate of increasing in carbohydrates should be remarkably higher in the later period of growing season when the elongation of shoots nearly ceased than in the early period when the shoots are growing very rapidly, and also the formation of flower buds should be affected more remarkably by pinching performed in the period of active growing than by that practised later when shoot elongation nearly ceases. However, neither is the case. Harvey's data (1) showed that the rate of increasing in total carbohydrates of apple shoots in the period after the active growth nearly ceased, was not higher than that in the period of rapid growing, and Kawaguchi (3) reported that in his studies with the Japanese pear trees that shoots headed in the

middle of May when they were growing rapidly, formed flower buds rarely, while many of the shoots headed at the end of June or in early July when their elongation declined or nearly ended, formed flower buds.

Conclusion

The results obtained in this investigation show that the most remarkable effect of pinching on the chemical changes in the shoots is the rather large increase of nitrogen. This suggests that in the case where flower bud formation is brought about by pinching, the increases of nitrogen should be, therefore, considered as a cause of flower bud formation, when it is interpreted from the point of carbohydrates and nitrogen relationships.

Literature Cited

- (1) HARVEY, E. M. A study of growth in summer shoots of the apple with special consideration of the role of carbohydrates and nitrogen. Oregon Agr. Exp. Sta. Bull. 200, 1923.
- (2) HOOKER, H. Changes produced in apple trees by various types of pruning. Missouri Agr. Exp. Sta. Res. Bull. 72, 1924.
- (3) KAWAGUCHI, M. Effects of summer pruning on development of secondary growth and flower bud formation of the Japanese pear shoots (in Japanese). Agriculture and Horticulture, 6: 563-569, 1929.