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CHANGES IN THE POLYSACCHARIDES OF THE TEA PLANT DURING POST-PRUNE GROWTH

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Key Word Index—*Camellia sinensis*; Theaceae; polysaccharides; reserves for shoot growth; starch; hemicelluloses.

Abstract—The ethanol-insoluble material (EIM) of the bark and wood of stems and roots, and developing shoots of the tea plant was fractionated and analysed. Detailed studies of the polysaccharide content of plants immediately after pruning and during recovery suggested that starch was the main reserve polysaccharide. Root wood contained the highest starch content on a dry weight basis. Recovery from pruning was accompanied by a decrease in the starch content in the root and stem. The analytical data were confirmed by histological studies. The holocellulose (hemicelluloses *A* and *B* and α-cellulose) content of the various organs showed negligible change during post-prune growth, suggesting that the hemicelluloses function only as structural material.

INTRODUCTION

THE PRACTICE of pruning in tea culture and the importance of root starch for post-prune growth have been described earlier.[1-3] Though it is evident that the carbohydrates utilized in early bud growth come mainly from reserves within the pruned plant, the exact nature and location of such reserves are not known with certainty. Tubbs[1] is of the opinion that roots are the chief storage organs, in the tea plant and that starch is the main storage form of carbohydrate. The root reserves, besides providing substrates for root respiration, appear to supply intermediates for the synthesis of various organic compounds which are translocated to developing shoots.[3] Movement of organic compounds from root to shoot during regeneration of lucerne has been demonstrated.[4]

It has been suggested that, in certain higher plants, hemicelluloses could also function as reserve material. However, the evidence for this hypothesis is meagre. Jermyn and Isherwood[5] studied changes in the cell-wall material of the pear during ripening and found that hemicelluloses behaved as part of the labile carbohydrate fraction. The seeds of leguminous plants are frequently rich in galactomannans, which function as food reserves.[6] The hemicelluloses found in the cell walls of the woody tissues of some trees also serve as a food reserve.[7] There is however, some conflict of opinion concerning the importance of hemicelluloses as reserve material.[8] A possible explanation is that hemicelluloses from different sources may differ in composition and that while some types of hemicellulose may function as reserves, others may function as structural material, or both. Because little or no information is available on the distribution and relative importance of the various groups of

[1] F. R. TUBBS, *J. Pomol.* **14,** 317 (1937).
[2] S. NAGARAJAH and U. PETHIYAGODA, *Tea Quart.* **36,** 88 (1965).
[3] R. R. SELVENDRAN, *Ann. Bot.* **34,** 825 (1970).
[4] K. C. HODGKINSON, *Austial J. Biol. Sci.* **22,** 1113 (1969).
[5] M. A. JERMYN and F. A. ISHERWOOD, *Biochem. J.* **64,** 123 (1956).
[6] E. PERCIVAL, *Comparative Phytochemistry* (edited by T. SWAIN), p. 152, Academic Press, London (1966).
[7] A. E. MURNEEK, *Plant Physiol.* **4,** 251 (1929).
[8] C. A. PRIESTLEY, *Tech. Commun. Bur. Hort. East Malling No.* 27, p. 13 (1962).

polysaccharide (starch, pectic substances, hemicelluloses and α-cellulose) of the tea plant in post-prune growth, an investigation was carried out to determine the changes they underwent during this period.

RESULTS AND DISCUSSION

Localization of Starch

Root-wood. Immediately after pruning the starch content was high in the inner xylem parenchyma, 40–50% of the cells being stained by the I_2/KI reagent. During post-prune growth, there was a centripetal decrease in the number of starch-containing cells. It was noted that most of the starch-depleted cells contained a number of granules which were not stained.

Stem-wood. Immediately after pruning all the peripheral cells of the pitch and a small proportion (10–15%) of the inner xylem parenchyma contained starch. During post-prune growth, the starch in the pitch was first depleted followed by a decrease in the xylem parenchyma. The starch-depleted cells contained a number of unstained granules as in root-wood.

TABLE 1. PERCENTAGE COMPOSITION OF HOT WATER-SOLUBLE POLYSACCHARIDES OF THE EIM OF ROOT-WOOD, ROOT-BARK, STEM-WOOD AND STEM-BARK OF THE TEA PLANT IMMEDIATELY AFTER PRUNING

Polysaccharides present	Tissues			
	Root-wood	Root-bark	Stem-wood	Stem-bark
Glucosan (Starch)	95	31·4	51·8	27·9
Galactan	0·8	18·1	7·7	17·3
Xylan	0·6	0·2	4·3	4·0
Araban	1·1	25·3	12·1	16·8
Polygalacturonic acid	1·1	24·9	8·9	33·0
Rhamnan	0·1	0·1	0·03	1·0
'Disaccharide'	1·3	—	14·9	—

Composition of Ethanol-Insoluble Material (EIM)

The sugars produced on hydrolysis of all the arbitrary fractions—hot water-soluble polysaccharides (Table 1), ammonium oxalatesoluble pectic acid, hemicellulose *A* and *B*, and α-cellulose—were glucose, galactose, xylose, arabinose, galacturonic acid and rhamnose (a trace). Mannose and fructose were not present, indicating that mannans and fructosans which are sometimes regarded as reserve material in plants were not present in any of the organs. A uronic acid was found to be present in the hemicellulose fractions but could not positively be identified on the paper chromatograms. Some disaccharides were detected in the hydrolysates of the α-cellulose and some of the hot water-soluble polysaccharide fractions; these are probably, incompletely hydrolysed cellobiose or 'similar units'. The composition of the ammonium oxalate-soluble pectic acid from stem and root bark was similar to the corresponding fraction from tea flush.[9] The composition of the hemicelluloses

[9] R. R. SELVENDRAN and B. P. M. PERERA, *Chem. & Ind.* p. 577 (1971).

and α-cellulose from the various organs compared well with the corresponding fractions from tea flush[9] and other plant tissues.[10,11]

The hot water-soluble fraction from the various organs gave a deep blue colour with the I_2/KI reagent suggesting the presence of starch in them. Because the EIM of the various organs (especially root-wood) was repeatedly extracted with hot water till the residue gave a negative test with the I_2/KI reagent, it may be inferred that most of the starch was extracted with hot water. The glucose produced on hydrolysis of the hot water-soluble fraction is assumed to be derived from starch. From Table 1, it is evident that the hot water-soluble fraction from root-wood contains the highest starch content relative to the other organs. Root-bark and stem-bark fractions contain substantial amounts of pectic substances.

The essential difference between the composition of the EIM of root and stem-wood is in the amount of hot water-soluble material (Table 2). The main component of the hot water-soluble fraction of root-wood was starch (Table 1). The quantity of $(NH_4)C_2O_4$-soluble pectic acid was negligible in root and stem-wood. The EIM of bark contained a higher percentage of pectic substances than wood.

TABLE 2. PERCENTAGE COMPOSITION OF THE EIM OF ROOT-WOOD, ROOT-BARK, STEM-WOOD, STEM-BARK AND DEVELOPING SHOOTS OF THE TEA PLANT IMMEDIATELY AFTER PRUNING (A) AND DURING RECOVERY (B)

	Tissues								
	Root-wood		Root-bark		Stem-wood		Stem-bark		
Constituent	A	B	A	B	A	B	A	B	Developing shoots*
Hot water soluble polysaccharides and proteins	23	9·5	11	18	4	2	19	14·7	25
Hot water soluble proteins	(0·6)	(0·8)	(0·6)	(0·7)	(0·3)	(0·4)	(0·9)	(0·7)	(2·6)
$(NH_4)_2C_2O_4$ soluble pectic acid	2·5	2	8	10	3	3·5	12	10	8
NaClO-soluble compounds	16	22	44	42	26	26·5	29	31·5	33
Holocellulose	58·5	66·5	36	30	67	66·5	40	42·5	34
Hemicellulose A	16	19	8	7	17	18	9	10	10
Hemicellulose B	16	10	5	5	16	11	5	3	8
α-Cellulose	29	36	21	17	36	39	28	31	16
EIM/Dry wt of tissue	0·93	0·91	0·58	0·62	0·94	0·96	0·75	0·8	43

B—the organs were collected from plants 56 days after pruning.
* The developing shoots were collected from plants 35 days after pruning.

Changes in the Composition of the EIM During Post-prune Growth

Root-wood and stem-wood. In root-wood, the main change accompanying recovery was the marked depletion in the starch content (Tables 2 and 3). These results are supported by the histological observations. The difference in the holocellulose (hemicelluloses *A* and *B* + α-cellulose) content of root and stem-wood during recovery was slight, suggesting that their hemicellulose components were not utilized for recovery. It should be noted that an

[10] J. P. THORNBER and D. H. NORTHCOTE, *Biochem. J.* **81,** 455 (1961).
[11] J. P. THORNBER and D. H. NORTHCOTE, *Biochem. J.* **82,** 340 (1962).

allowance has to be made for the high content of the starch of root-wood (hot water-soluble fraction) when calculating its holocellulose content from the EIM.

TABLE 3. THE STARCH CONTENT OF THE VARIOUS ORGANS OF THE TEA PLANT IMMEDIATELY AFTER PRUNING (A) AND DURING RECOVERY (B)

Tissue	Starch content (mg glucose/g dry wt) A	B
Root-wood	180	30
Root-bark	54	8
Stem-wood	32	7
Stem-bark	49	13

B—56 days after pruning.

The average dry wt of the various organs of a pruned plant was as follows. Root-wood–36 g, root-bark–8 g, stem-wood–32 g and stem-bark–5 g.

Root-bark and stem-bark. The EIM of root-bark and stem-bark contained adsorbed polyphenols, particularly root-bark after recovery, an observation which is reflected in the higher content of its hot water-soluble fraction. Most of the adsorbed polyphenols of the EIM were removed by hot water and the remainder was completely removed during the delignification step. The starch content of the root-bark and stem-bark decreased during the recovery period (Table 3), but the holocellulose showed negligible change. It should be noted that in the EIM of root-bark after recovery, an allowance has to be made for the increased adsorption of polyphenols when calculating its holocellulose content.

Developing shoots

The composition of the EIM of the developing shoots compare well with that of the tea flush.[9] The presence of starch in the stem-bark and stem-wood of recovering plants (Table 3) suggests that part of the stem reserves may be utilized to initiate and support the growth of new shoots. Further, experiments with $^{14}CO_2$ showed that even the mature brown stems of pruned plants are capable of fixing carbon dioxide into soluble and insoluble constituents in the presence of light (Selvendran, unpublished results). It is possible that some of these constituents are also utilized for new growth. It therefore appears that the developing shoots synthesize their cell-wall and cytoplasmic material from stem reserves (starch), photosynthetically elaborated compounds and material translocated from the roots.[3,4] The hemicelluloses of the stem-bark and stem-wood are probably not utilized by the developing shoots.

The results of this investigation support postulate of Tubbs[1] that starch is the main reserve polysaccharide of the tea plant and that it is stored in the root. During the recovery period, the root reserves (root-wood and root-bark) may be respired away, translocated into fine lateral roots or into developing shoots. Using acidic dyes like fuschin and eosin and ^{14}C labelled L-leucine and D-glucose, evidence was obtained for translocation of material from the roots to the aerial parts of the plant during the recovery period.[12] These findings support the hypothesis that part of the root reserves are mobilized and translocated to sup-

[12] R. R. SELVENDRAN and S. SELVENDRAN, *Proceedings of the Ceylon Association for the Advancement of Science*, Part 1, p. 79 (1971).

port the growth of new shoots.[3] As mentioned earlier, the starch present in the stem-bark and stem-wood may also be utilized to initiate and support the growth of new shoots.

The hemicelluloses of the various organs do not appear to function as reserve food and it may be that in the tea plant they function only as structural material.

EXPERIMENTAL

Plant material. All material (bark and wood of stems and roots and developing shoots) used in this investigation was collected from 2-yr-old plants (Clone Ken 16/3) grown in a field near the laboratory (1500 m elevation). The plants were clean-pruned on 4 August 1970 and stem and root samples were collected from the pruned plants at 0, 21, 35, 56, 70 and 84 days for histological studies. For detailed analysis stem and root samples (1–1·5 cm thick), samples were collected from three comparable plants immediately after pruning, and after 56 days. Developing shoots for analysis were collected 35 days after pruning. The sampling dates were decided on the basis of the growth stage of the new shoots on the frame.

The general vegetative conditions of the pruned plants, that is presence or absence of buds, leaves and shoots, at the time of uprooting were noted and were similar to those reported earlier.[3]

Histological studies. Transverse and longitudinal sections of stem and root (1–1·5 cm thick) were made from the samples collected. The sections were stained sequentially with 1% saffranin in 50% EtOH and I_2/KI reagent. Cellular accumulation of starch was indicated by development of a deep blue colour.

Fractionation and analysis of the ethanol-insoluble material (EIM). The methods used for the preparation, fractionation and analysis of the EIM were similar to those described earlier.[5,9,13] After each fractionation, the quantitative yield of residue was determined and aliquots taken for dry wt. None of these fractions was corrected for the mineral matter present. As the aldopentoses, aldohexoses and uronic acids have different reducing values when determined by the Park and Johnson method,[14] separate standard graphs were prepared for each group of sugars using xylose, glucose and galacturonic acid as standards. The protein content of the EIM was calculated from nitrogen values[15] and starch was determined by the method of Hassid and Neufield.[16]

The determination of the total available carbohydrates (TAC) of tea roots. The TAC of tea roots was determined by Nagarajah and Pethiyagoda[2] by hydrolysing tea root powder with 1 N H_2SO_4 for 2 hr and determining the reducing value of the hydrolysate. As hydrolysis under these conditions may liberate reducing sugars from compounds besides starch and sucrose (pectic substances, hemicelluloses, etc.), some experiments were carried out to determine the relative proportions of the sugars liberated on hydrolysis of the root-wood powder and the EIM of root-wood under these conditions. The sugars liberated from these material were similar. Glucose, xylose, galactose and arabinose were present in the hydrolysates of the root-wood powder in the proportions 83:12:3:2 respectively. From this it would seem that some caution has to be exercised in interpreting the results of the TAC when determined by the above method, especially when the starch content of the tissue is low.

[13] R. R. SELVENDRAN and B. P. M. PERERA, *Tea Quart.* **42,** 16 (1971).
[14] J. T. PARK and M. T. JOHNSON, *J. Biol. Chem.* **181,** 149 (1949).
[15] A. C. CHIBNALL, M. W. REES and E. F. WILLIAMS, *Biochem. J.* **37,** 354 (1943).
[16] W. Z. HASSID and E. F. NEUFELD, *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER), Vol. 4, p. 33, Academic Press, New York (1964).