130. THE CONSTITUTION OF THE CAMBIUM, THE NEW WOOD AND THE MATURE SAPWOOD OF THE COMMON ASH, THE COMMON ELM AND THE SCOTCH PINE

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The composition of the mature cell wall of many plant tissues has been investigated in considerable detail, but there is little information as to the chemical nature of the wall in the earlier stages of its development. This is particularly so in woody plants in which the mature wood has been the subject of extensive research, while the study of the developing tissue has been almost completely neglected. Possibly one of the reasons for this neglect is the apparent difficulty of obtaining the necessary material. In the present work, it was found that the cambium and the differentiating xylem produced from it could be collected without great difficulty in amounts which permitted the determination of the main cell wall constituents.

There have been several previous analyses of the cambial sap of trees [Wislicenus, 1928; Schwalbe & Neumann, 1930; Wislicenus & Hempel, 1933] but this work was concerned with the water-soluble constituents of the sap and not with the insoluble matter of the cell wall. The cambial sap was obtained by removing the bark from the tree in early summer and scraping either the inner surface of the bark or the exposed surface of the wood. In the work of Schwalbe & Neumann [1930] and in the earlier work of Wislicenus [1928], the scraping was carried out by broken pieces of glass or porcelain. This method has the disadvantage of removing some of the tissues of the outermost sapwood, which together with the sap from the injured vessels are mixed with the tissues of the cambium and may give rise to misleading results. Even if the mature vessels are not ruptured, the so-called cambial sap will include differentiating xylem elements if collected from the surface of the xylem, and differentiating phloem elements if obtained from the inner surface of the bark. It is desirable that the two types of sap should not be mixed and in any work on the development of the wood only the cambium on the surface of the xylem should be investigated. Since the results are influenced to such an extent by the method of preparation, in investigations on the cambial sap the source of the sap should be clearly indicated. In his interesting work on the pectic substances of *Robinia*, Anderson [1936] dealt with the "cambium-phloem" layer, but from the description in the published paper it is not certain what tissues are included in this material.

In the present work, the material described as cambium includes a proportion of the true cambium and also the soft-walled differentiating xylem elements immediately within the cambium proper. The new wood comprises the xylem elements already produced from the cambium during the current year. For purposes of comparison the mature sapwood of the three species was also investigated.

Experimental

Methods

Material. The three species used included two hardwoods, namely the common ash, *Fraxinus elatior* L., and the common elm, *Ulmus sativa* Mill., and one softwood, the Scotch pine, *Pinus sylvestris* L. The material was collected from the hardwoods during the second week of May 1939, and from the Scotch pine, in which cambial activity is renewed at a later date, on 30 May. In each case well-grown trees were used.

Our method of obtaining the cambium differed only in detail from that employed by previous workers [Schwalbe & Neumann, 1930; Wislicenus & Hempel, 1933]. It is well known that with the resumption of cambial activity in spring, the bark readily "slips" from the woody core, the separation occurring at the cambial layer. The cambium and the differentiating xylem can then be scraped from the exposed surface of the wood. By using the rounded edge of a spoon to remove the soft developing tissue we avoided the inclusion of parts of the mature wood. After complete removal of the softer tissues it was found that the fully formed wood which had already been produced from the cambium during the current year could easily be separated from the late wood of the previous year with the aid of a slightly blunted chisel. In the ash there was a sharp transition between the gelatinous differentiating tissue and the more mature elements already produced; in the elm the distinction was not so marked, while in the pine the more uniform tissue could not be separated into cambium and new wood. The sapwood samples of each tree were obtained from the outer annual rings after removal of the cambium and the newly formed wood.

Treatment of fresh material. The cambium and the new wood of the three species were each dropped into boiling 95% alcohol to give a final concentration of not less than 80% alcohol. After refluxing for several hours, the alcohol was cooled and filtered off and the residue re-extracted with 80% alcohol. The process was repeated until the extraction was complete. The extracted tissue was dried in the air and ground in a mortar to pass a 60-mesh sieve.

In each case the sapwood was air-dried and ground in a mill to pass a 60-mesh sieve. The powder was extracted with alcohol as for the cambium and new wood and again spread out to dry in the air.

Analytical methods

(*a*) *Water content.* The amount of water in the fresh material was determined by drying a sample in an electric oven at 105°. The amount of dry matter in the air-dried alcohol-extracted material was obtained in the same way.

(*b*) *Ash.* The ash content was determined by heating the residue from (*a*) in an electric muffle furnace at dull red heat.

(*c*) *Alcohol-soluble matter.* An aliquot of the 80% alcohol extract was evaporated on a steam bath and drying completed in the oven at 105°.

(*d*) *Reducing substances.* After boiling off the alcohol, the reducing power of an aliquot of the alcohol extract was determined using the modifications adopted by van der Plank [1936].

(*e*) *Sucrose.* The hydrolysis of sucrose was effected by invertase.

All the following determinations were carried out on air-dried alcohol-extracted material, a correction being made for moisture content.

(*f*) *Nitrogen.* The total N was determined by the Kjeldahl method and expressed as protein after multiplication by the factor 6·25.

(*g*) *Cellulose.* Norman & Jenkins' [1933] modification of the Cross and Bevan process was adopted.

(*h*) *Lignin.* The material was digested with 72% H_2SO_4 for 16 hr., diluted to 3% and boiled for 2 hr. Although the product is not pure it can usually be taken to be a measure of the lignin content.

(*i*) *Furfuraldehyde.* The furfuraldehyde yield was determined by distillation with 12% HCl and precipitation as phloroglucide, using the modifications suggested by Angell *et al.* [1936]. Determinations were also carried out on the cellulose obtained in (*g*). The dried cambium cellulose could not be dispersed in the acid and in this case a fresh preparation was washed directly into the reaction flask.

(*j*) *Uronic acid.* The procedure of Dickson *et al.* [1930] was followed. Determinations were carried out before and after extraction with 0·5% ammonium oxalate, the loss in uronic acid after extraction yielding a value for the total pectin content of the material.

(*k*) *Mannan.* Determinations of mannan were restricted to the softwood material. Nowotnowna's [1936] method was employed.

Results

The results of determinations on the fresh material and on the alcohol extract are set out in Table 1, while Table 2 contains the results of determinations on the alcohol-extracted material.

Table 1. *Compositions of cambium, newly formed wood and mature sapwood of common ash, common elm and Scotch pine*

	Water as % fresh material	Dry matter as % fresh material	Ash as % dry matter	Alcohol-extractable material as % dry matter	Reducing substances as % dry matter	Sucrose as % dry matter
Ash:						
Cambium	88·5	11·5	10·4	74·7	3·22	26·8
New wood	74·3	25·7	3·85	27·6	1·73	6·92
Sapwood	34·7	65·3	0·49	5·83	0·26	1·35
Elm:						
Cambium	90·55	9·45	20·1	47·7	2·27	11·2
New wood	81·2	18·8	7·27	29·0	2·16	1·66
Sapwood	47·8	52·2	0·48	5·66	0·59	0·21
Pine:						
Cambium	86·0	14·0	6·11	62·0	10·2	27·2
Sapwood	48·5	51·5	0·22	3·20	0·41	0·32

In each species the water content of the cambium is high, as would be expected in young actively growing tissue. The new wood of ash and elm has a much higher water content than the older sapwood.

The ash contents of the three cambium samples are high when expressed as percentages of the dry matter, but form only small proportions of the fresh weights. The amount of mineral matter in the old wood is low, while that of the new wood lies between the figures for the cambium and old wood.

In the cambium of all three species the alcohol-soluble part forms a high proportion of the dry matter, especially in ash. This fraction is only small in the old sapwood, but exceeds 25% of the dry weight in the newly formed wood.

The free reducing substances formed only a comparatively small proportion of the alcohol extract of the cambium, which had, however, a much higher

sucrose content. Klason [1929] also found a high concentration of sucrose in the cambial sap of the fir, while Wislicenus [1928; Wislicenus & Hempel, 1933] states that invert sugar forms the main part of the dissolved substance. The latter result could be obtained by the action of an enzyme during the preparation of an aqueous extract of the sap. In the present work activity of enzymes was avoided by dropping the freshly collected cambium into boiling alcohol. The sugar content of the new wood is much lower than that of the cambium, but it should be pointed out that some of the sugar in the cambium has possibly escaped from the vessels of the new wood. As might be expected, there is little sugar in the older sapwood.

Since we were mainly interested in the cell-wall substances no determinations were made of the other components of the alcohol extract, which also includes glucosides, lipins, soluble N etc.

The residue from the alcohol extraction contains the main constituents of the cell wall and was analysed in greater detail than the alcohol extract.

Tests for starch with iodine solution were negative for all samples of the cambium and new wood; the older sapwoods of elm and pine gave only slight colorations, while a strong starch reaction was obtained in ash sapwood. These results were to be expected since it is not likely that starch would accumulate in young actively growing tissue.

Since all the cells of the cambium region have living contents, the high N obtained for the cambium is in accordance with expectation. The new wood has a higher N content than the older sapwood.

The percentage of cellulose is considerably lower in the cambium than in the new wood or sapwood. In both ash and elm the cellulose content of the new wood is less than that of the older sapwood. The lower value of the new wood may be in part a result of incomplete development, or may be explained wholly by the difference in composition known to exist between the early and late woods of the single annual ring [Ritter & Fleck, 1926; Preston & Allsopp, 1939]. The cellulose obtained from the new wood and older sapwood was white and fibrous, while that of the cambium was grey and horny. A specimen of the cellulose from ash cambium was examined by Dr R. D. Preston, of this Department, who found that the X-ray diffraction pattern was closely similar to that of cotton cellulose.

The furfuraldehyde produced from the cellulose arises from the cellulosans which in the hardwoods are built up almost entirely from xylose [Norman, 1937, 1, p. 20]. In ash and elm the xylan content of the cambium cellulose is greater than that of the sapwood, but less than that of the newly formed wood. The high proportion of xylan in the cambium cellulose is of interest in suggesting that the xylan arises early in the life of the cell, and is probably not secondarily produced from the cellulose. The lower xylan content of the sapwood as compared with the new wood may again be explained by the fall in pentosan content from early to late wood [Ritter & Fleck, 1926; Preston & Allsopp, 1939], but in the ash the difference is of such magnitude as to suggest that other factors might operate, as for example, a change in composition subsequent to vessel differentiation. The high xylan figure of the ash new wood is in excess of the value previously recorded for other wood celluloses. The mature sapwood of pine like that of the other species has a lower xylan content than the cambium. In addition to xylan, mannan is found in the cellulose of conifers. The proportion is surprisingly high in the cambium of pine and is far in excess of the amount in the sapwood cellulose.

The lignin values for the mature woods of the three trees merely confirm the previous analyses of this material. The new woods of ash and elm are again of

interest in the possession of higher lignin figures than the sapwood. In this case it is almost certain that the difference reflects that normally observed between early and late woods. Ash cambium which was the purest of the three species has a very low lignin content, the elm cambium has a higher lignin content, part of which is certainly derived from small amounts of the new wood present as impurity, while the pine cambium has a relatively low lignin content. Although the lignin values for the differentiated wood are reasonably accurate, there are probably fairly large errors in the determinations on the cambium, where there is a high concentration of substances such as proteins, pentoses etc. which increase the yield of apparent lignin [Norman, 1937, 2]. But although the figures are not precise they do indicate that the material used in the present investigation consists of relatively unlignified tissue.

The preparation of the cellulose by Norman & Jenkins' method [1933] supplied further information of the nature of the lignin. It is well known that the lignin of gymnosperm woods differs from that of angiosperm woods in yielding a brownish colour instead of magenta when warmed with sodium sulphite solution after treatment with chlorine. In the present work we were able to extend this observation to the cambium of the species investigated. Following two hypochlorite treatments the usual treatment with chlorine and sodium sulphite resulted in a magenta colour with ash and elm cambium and a brown colour with that of pine. This treatment removed all the lignin from the cambium and no coloration was produced after a second chlorination.

The newly differentiated woods gave higher lignin values than the mature wood, but it would seem that the polymerization of the lignin units is of a lower grade in the former, since the new woods of ash and elm were delignified after 4 chlorinations while the mature woods required 7 and 9 chlorinations respectively. Pine wood required 8 chlorine treatments.

The pectin values given in Table 2 are obtained by multiplying the fall in CO_2 output after extraction with 0·5% ammonium oxalate by the factor 5·66.

Table 2. *The compositions of the cambium, newly-formed wood and mature sapwood of common ash, common elm and Scotch pine*

Material ...	Ash			Elm			Pine	
	Cambium	New wood	Sapwood	Cambium	New wood	Sapwood	Cambium	Sapwood
Cellulose	20·2	51·9	58·3	20·7	53·0	57·5	25·1	61·8
Lignin	4·60	23·2	20·9	13·5	25·5	24·8	8·56	26·1
Pectin	21·6	3·85	1·58	9·91	3·72	1·68	16·6	0·96
Protein	29·4	5·56	1·37	30·0	5·19	1·73	20·8	0·83
Total furfuraldehyde	10·6	17·3	15·2	10·3	15·1	14·2	13·0	6·42
Furfuraldehyde not in cellulose	7·04	6·10	6·16	6·64	5·00	5·52	10·67	3·49
Xylan in cellulose as % cellulose	26·9	33·7	24·5	26·3	29·8	23·8	15·0	7·9
Mannan in cellulose as % cellulose	—	—	—	—	—	—	21·9	6·16
Total uronic anhydride	20·2	8·20	5·25	12·5	7·86	6·44	15.7	3·71
Uronic anhydride not extracted by ammonium oxalate	4·90	5·48	4·10	4·6	5·23	5·25	4·00	3·03
Furfuraldehyde in hemicellulose	1·66	4·13	4·95	3·66	3·12	4·04	6·48	2·64

The high values recorded for ash and pine cambium are very striking, while elm cambium has also a relatively high pectin content. It is clear that the wood contains at most only a small amount of pectin, especially in pine. The quantity in the new wood is still low but higher than that of the mature wood.

Our results are in general agreement with the more detailed investigations on the pectic substances of woody materials by Anderson and his co-workers [1936; 1937]. These workers also find that conifer wood has a lower pectin

content than hardwoods, while their "cambium-phloem" had a high pectin content, although the values did not attain the magnitude of those obtained in the present work.

At the present time there is unfortunately no satisfactory method of determining hemicelluloses, but a consideration of the furfuraldehyde and uronic anhydride contents affords some information as to the nature and amount of this fraction. The non-cellulosic furfuraldehyde arises from the pentoses and uronic acids contained in pectin and in the hemicelluloses. The furfuraldehyde yield of the uronic acids is known [Angell *et al.* 1936] and many analyses of pectin preparations have shown that the furfuraldehyde yield is never far from 20% [Norman, 1937, 1, p. 80]. Since the pectin and the hemicellulose uronic anhydride have been determined, subtraction of the calculated furfuraldehyde yield of these constituents from the total non-cellulosic furfuraldehyde affords an approximate measure of the furfuraldehyde produced from the pentosans of the hemicelluloses. The figures obtained in this way are set out in Table 2 as "furfuraldehyde in hemicellulose". It should be mentioned that in our calculations we have made the assumption that we are concerned only with one uronic acid. The error involved is considerable, but like that arising from the fact that the furfuraldehyde yield of a mixture is not the sum of the furfuraldehyde yields of the constituents [Angell *et al.* 1936] is not sufficiently great to affect the general conclusions. The differences are enough to show that ash cambium has a far lower pentose content than either the new or mature wood. This is not the case for the less pure cambium of elm, but the other analyses for elm cambium are also characteristic of a more mature material than those of ash cambium. The pine is clearly quite a different case, the cambium containing far more pentose than the mature wood. It would seem that in ash and elm the differences between new and mature woods are sufficiently great to warrant the statement that the mature wood has a higher pentose content than the newly formed wood. The results show a higher uronic acid:pentose ratio than that obtained in isolated hemicellulose preparations [Norman, 1937, 1, p. 40]. This discrepancy can be explained by a failure to remove all the pectin from our samples before determination of hemicellulose uronic anhydride. It is also possible that the lower ratio in isolated hemicelluloses might be accounted for by degradation of uronic acids during extraction.

Discussion

The analytical results described above reveal that the cambium and differentiating xylem of ash, elm and pine have similarities in the composition of the cell wall with other young tissues. The high pectin content is particularly characteristic of the younger stages of the cell wall [Buston, 1935; Griffioen, 1938; 1939]. It is in this respect, and also in the lower lignin:cellulose ratio that the cambium and differentiating elements differ chiefly from the mature wood. The analyses of the newly formed wood supplied some evidence that even after vessel differentiation small changes in composition take place involving a further loss of pectin, a small increase in the amount of encrusting pentosans and an increase in the resistance of the lignin components.

Several hypotheses of the origin of the constituents of mature tissues have been based on a consideration of the composition of younger tissues. A fall in the proportion of one component accompanied by an increase in that of another has been cited as evidence of the production of the second from the first substance. In this manner, the great fall in concentration of pectin which occurs during the development of lignified tissues has been taken to indicate that the lignin is formed from the pectic constituents [Griffioen, 1938; 1939; O'Dwyer, 1932]. Our

results might be claimed to support this view, but it should be emphasized that the change in the concentration of pectin in passing from the cambium to the mature wood is no evidence of an alteration in the total quantity. During the development of the xylem elements the thin walls of the differentiating cells undergo a great increase in thickness and it is probable that little or no pectin is deposited in the additional thickening layers. If the pectin present in the younger stages persisted unchanged, the total amount would undergo no alteration while the concentration would be greatly reduced. This view is supported by the work of Buston [1935] who found no fall in the pectin content during the lignification of rose shoots, although the concentration was considerably reduced. Even if the pectin were reduced in amount it would not necessarily imply that it had undergone transformation into another cell wall constituent.

In the ash cambium a large amount of pectin is accompanied by a small amount of encrusting pentosan. Such evidence is used in support of the view that the encrusting hemicelluloses arise from pectins, but the change in proportion of these constituents can equally readily be explained by an alteration in the composition of the later thickening layers of the cell wall. Several workers have suggested that the encrusting pentosans afford a source of lignin and in this connexion it may be of some significance that in the cambium of pine the pentosan content is unusually high but is quite low in the mature wood. More detailed work on the composition of the hemicelluloses of cambium is desirable and might throw further light on the origin and subsequent fate of these comparatively neglected cell-wall constituents.

Summary

The woods of the common ash, common elm and Scotch pine were divided into three fractions: cambium + differentiating xylem, newly formed wood and mature sapwood. Analyses were made of each fraction.

The composition of the cambium was found to be similar to that of other differentiating tissues especially in the high pectin and relatively low lignin contents. There is some evidence that further slight changes occur in the composition of the wall after vessel differentiation is complete.

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