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LIGNIN STRUCTURE IN *BUXUS SEMPERVIRENS* REACTION WOOD

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Key Word Index—*Buxus sempervirens*; Euphorbiaceae; box; compression wood; growth stress; lignin; growth strain measurement; thioacidolysis.

Abstract—The chemical structure of lignins in *Buxus sempervirens* wood samples showing a progressive grading from normal wood to reaction wood was investigated by thioacidolysis. The mechanical states of the various samples were assessed quantitatively by measuring the growth strains at the stem surface. Sample woods with the more pronounced compression character showed the highest lignin contents. *Buxus* lignins are composed of guaiacyl and syringyl units, similar to more evolved angiosperm lignins. The proportion of syringyl units, together with that of labile inter-unit ether bonds, was, however, lower in boxwood lignins than in poplar or birch lignins. More importantly, lignins in boxwood samples with a pronounced compressed character were found to have structural similarities with gymnosperm compression wood lignins, i.e. a higher content of carbon–carbon inter-unit bonds and of *p*-hydroxyphenyl units, compared with normal wood lignins. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Compression wood, induced on the lower side of leaning stems, occurs almost exclusively in gymnosperms [1, 2]. It is also present in some primitive woody angiosperms with tracheids as predominant xylem cells [3]. Thus, members of the genus *Buxus* have been reported to form reaction wood with various features resembling those of gymnosperm compression wood [3–5]. However, as *Buxus* reaction wood lacks some anatomical features of gymnosperm compression wood, Timell [1] pointed out that more information is required before its nature can be fully assessed. In a recent microspectrophotometric study, Yoshizawa *et al.* [6] observed that the secondary walls of *Buxus* reaction wood are characterized by excessive lignification and that the corresponding lignins, composed of guaiacyl (G) and syringyl (S) units, are relatively enriched in G units, compared with normal wood lignins. The proportion of *p*-hydroxyphenyl (H) units, which typically increases in gymnosperm compression wood lignins [1], was, however, not addressed in this cytochemical investigation [6].

In the present work, we examined the chemical structure of lignins in *B. sempervirens* wood samples showing a progressive grading from normal wood to reaction wood. This chemical investigation was performed by thioacidolysis, a specific lignin degradation method, which provides a signature of the H, G and/or S building units involved in labile ether bonds [7]. Because the occurrence of typical anatomical markers of compression wood is still the subject of controversy [1], we unambiguously assessed the mechanical states of the various wood samples by measuring the growth strains at the stem surface [8].

RESULTS AND DISCUSSION

A series of six boxwood specimens (nos 1–6), corresponding to a progressive transition from normal wood to compression wood, was collected from different levels of crooked stems and characterized on the basis of mechanical measurements. In addition, two other samples were taken from the lower (sample C) and upper (sample N) sides of a leaning stem with a dark colour and a pronounced growth eccentricity toward the lower side. On this basis, sample C could be considered as a typical compression wood sample and sample N could be used as a reference for normal wood.

Growth stresses originate in surface growth strains induced in the cambial layer during the differentiation and maturation of new cells and impeded by the mass of the whole trunk. The longitudinal growth strains at the stem surface of the boxwood samples were determined by means of an extensometric sensor, which measures the surface stress release obtained by sawing two grooves in the wood under the cambium [8] (see Experimental). The microstrain values reported in

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Table 1. Average growth strain values and lignin contents of a series of *Buxus sempervirens* samples (1–6) showing a progressive gradation from normal to compression wood. Samples N and C are reference woods taken from the upper and lower side of a leaning stem, respectively. This leaning stem showed a dark colour and a pronounced eccentricity of growth toward the lower side, a characteristic of severe compression wood [1]

	Growth strain ($\mu m\ m^{-1}$)	Lignin content*
N	−635	27.9
1	−741	27.8
2	−159	27.0
3	184	26.8
4	616	27.4
5	1211	27.3
6	2018	29.0
C	2782	31.0

*Expressed as weight percentage of Klason lignin in extractive-free wood meal.

Table 1 assess the mechanical state of the various *Buxus* specimens on the following rationale. A compression stress (as in compression wood) induces a swelling when released and, thereby, a positive longitudinal growth strain (expressed in $\mu m\ m^{-1}$). The mechanical diagnostic of compression wood is, therefore, a high positive value of growth strain. In contrast, normal wood shows low or even negative growth strain values. Samples 1–6 showed a progressive transition from normal to compression wood, the higher growth strain value corresponding to sample 6. Sample C, with pronounced macroscopic features of compression wood, was also characterized by a large growth strain value (+2782) (Table 1).

The lignin contents of the various boxwood samples were determined from their extractive-free sawdust. Although there is not a direct correlation between lignin content and growth strain values, the data of Table 1 suggests the following trend: the markedly higher lignin contents correspond to the wood samples with pronounced compression character (samples 6 and C). Similar to gymnosperm compression wood, the reaction wood formed in the lower side of inclined boxwood stems seems to contain both a higher lignin content and compressive stress. The lignin enrichment is, however, less pronounced than in the case of gymnosperm compression wood [1].

The main aromatic monomers recovered from the thioacidolysis of the boxwood samples were identified after silylation by GC-mass spectrometry and quantified by GC [7]. Their absolute yield and relative distribution are included in Table 2. For comparison purposes, similar data obtained for more evolved angiosperm woods and for gymnosperm woods are also reported in Table 2. The H, G and S monomers originate from the H, G and S units involved in labile ether bonds (β-O-4 bonds). The data show that the occurrence of β-O-4 linked G and S units is a structural feature common to this primitive angiosperm and to more evolved angiosperm woods. However, the boxwood samples show a markedly lower content in S units than poplar or birch lignins. The yield of the thioacidolysis aromatic monomers is about half of that obtained from more evolved angiosperm wood and is similar to that of gymnosperm woods (Table 2). This indicates that the β-O-4 substructures, which specifically generate these monomers, are fewer in the *Buxus* primitive wood than in more evolved angiosperm woods. Conversely, boxwood lignins are richer in carbon–carbon inter-unit bonds than are poplar or birch lignins.

The proportion of G units, which give rise to G thioacidolysis H monomers, is increased in boxwood

Table 2. Yields and relative distribution of H, G and S monomers released from the thioacidolysis of extractive-free *Buxus* samples showing a progressive gradation from normal to compression wood (1–6). Samples C and N correspond to typical compression and normal boxwood samples (see Table 1). The data for more evolved angiosperm (poplar and birch) wood lignins and for gymnosperm (spruce wood and pine compression wood) are shown for comparison

Sample	Total yield H + G + S ($\mu mol\ g^{-1}$ Klason lignin)	Relative molar distribution H/G/S
Buxus samples		
N	1262	tr/67/33
1	1491	tr/66/34
2	1484	tr/64/36
3	1458	tr/65/35
4	1398	tr/67/33
5	1401	2/69/29
6	1226	3/71/26
C	947	3/71/26
Other species		
Poplar (*Populus euramericana*)	2390	nd/41/59
Birch (*Betula verrucosa*)	2460	nd/24/76
Spruce (*Picea abies*)	1230	2/98/tr
Pine (*Pinus pinaster*) compression wood	1140	18/82/tr

tr: traces; nd: not detected.

samples with the more severe compression character (samples 6 and C, Table 2). This quantitative evaluation is consistent with qualitative microspectrophotometric data previously reported [6]. Concomitantly to the enrichment in G units, there is a decrease in the overall thioacidolysis yield. This decrease is indicative of a higher content of resistant carbon–carbon bonds which are not analysed by thioacidolysis.

Compression wood lignin is generally reported to be much richer in H units than normal gymnosperm wood lignin [1, 9], as exemplified in Table 2. The proportion of H units in the boxwood samples was specifically investigated by thioacidolysis. We observed that the proportion of H units increases from trace amounts to a few per cent during the change from normal to compression *Buxus* wood. To assess this tendency better and to convert data referred to as trace amounts in Table 2 into quantitative values, mass chromatograms were obtained by selectively monitoring the most specific ion of the trimethylsilyl thioacidolysis H monomer, at *m/z* 239 (base peak). The relative surfaces of this selected ion were comparatively measured for the various boxwood samples. With a reference value arbitrarily set at 100 for the H signals in samples 6 and C, the H relative contents of samples 5, 4, 3, 2, 1 and N were 46, 33, 10, 8, 7 and 4, respectively. These data confirm that the gradual change from normal to compression wood in *Buxus* is accompanied by an enrichment in H lignin units. This feature is therefore common to *Buxus* compression wood and to gymnosperm compression wood [9].

EXPERIMENTAL

Plant material. Wood specimens were obtained from old trees of *B. sempervirens* L., 4 m high, growing in the southeast of France near Montpellier. Fifteen trees were selected from different levels of leaned stems for 36 growth strain measurements corresponding to a progressive transition from normal to compression wood. For chemical analysis, samples were divided into a series of six classes (1–6), each including five or six samples exactly taken between the grooves (Table 3). For each class, mean growth strain was calcd, weighed by the mass of each respective withdrawal (*ca* 0.4 g). In addition, two other samples were taken from the lower (sample C) and upper (sample N) sides of a highly crooked stem with a dark colour and a pronounced growth eccentricity toward the lower side.

Growth strain measurements. Longitudinal growth strains at the stem surface was appraised by stresses release on the stem periphery, as previously described [8]. The technique for measuring the longitudinal growth strains at the stem surface used an extensometric sensor (manufacturer HBM, type DD1). The total longitudinal stress was determined by sawing two

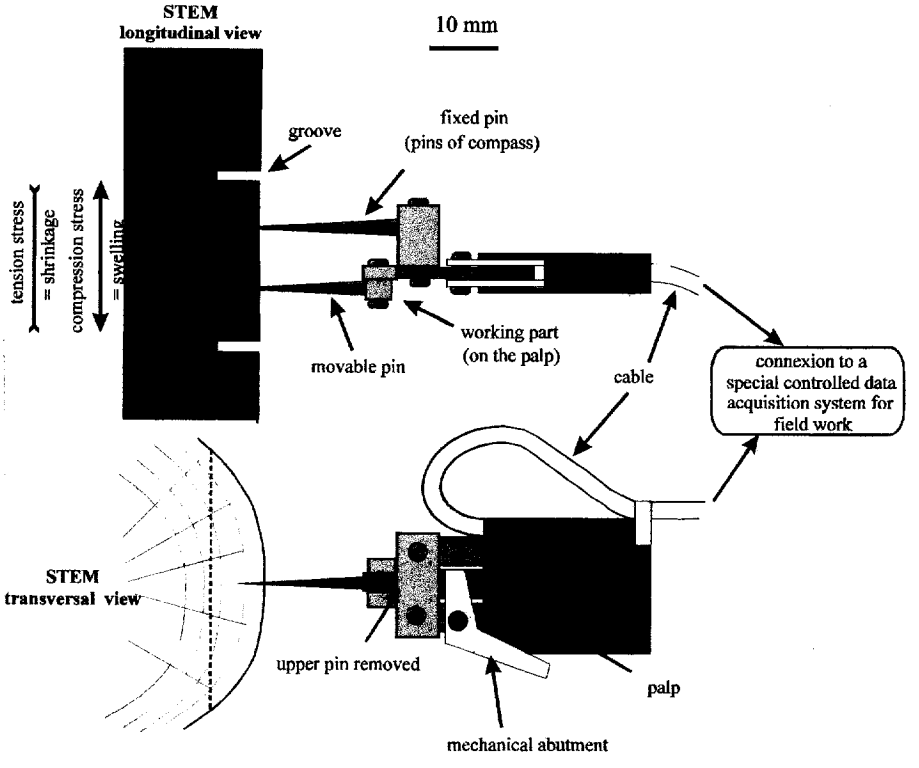

Fig. 1. Measurement of longitudinal growth strain with an extensometric sensor. Total longitudinal stress is released by sawing two grooves above and below the sensor. After this operation the longitudinal growth strain is achieved and expressed in microstrain (in $\mu m\ m^{-1}$).

Table 3. Sampling protocol for the six classes of *Buxus* samples

Tree (letter) and measure (number) references	Dry weight (DW) (g)	Growth strain (GS) (μm m^{-1})	Total weight (TW) (g)	Sample and weighed growth strain (WGS) (μm m^{-1})	
A5	0.321	−1400			
A6	0.364	−1246			
G7	0.342	−630	2.321	No. 1	−741
G1	0.468	−560			
H2	0.452	−448			
M8	0.374	−364			
L3	0.422	−280			
H3	0.364	−196			
H4	0.211	−140	2.340	No. 2	−159
I4	0.418	−98			
P9	0.464	−98			
L4	0.461	0			
D5	0.313	84			
H7	0.363	98			
H5	0.375	196	1.705	No. 3	184
G4	0.397	252			
N6	0.257	308			
N4	0.330	420			
O4	0.493	504			
J1	0.384	588	2.077	No. 4	616
Q6	0.394	742			
D1	0.476	784			
P8	0.412	938			
P1	0.359	1078			
S2	0.368	1176			
G3	0.373	1190	2.307	No. 5	1211
S7	0.350	1400			
J3	0.445	1470			
F2	0.466	1596			
A7	0.415	1610			
A1	0.388	1988	2.377	No. 6	2018
C6	0.408	2128			
N8	0.400	2296			
G8	0.300	2758			

$$GWS = \frac{1}{TW} \sum_i DW_i.GS_i .$$

grooves above and below the sensor (Fig. 1). This cutting is assumed to release locally existing stresses in the stem. The observed strains are, therefore, proportional and have opposite signs to the initial stresses. After this operation, we measured the longitudinal growth strain expressed in microstrain (in μm m^{-1}). A compression stress (as in compression wood) induces a swelling between the grooves; thus, the longitudinal growth strain is positive. A tension stress (as in tension wood or in normal wood) induces a shrinkage between the grooves; thus, the longitudinal growth strain is negative.

Thioacidolysis. Thioacidolyses of extractive-free boxwood samples and GC quantitative analyses of the TMSi reaction products were carried out as previously described [7]. GC-MS identification of the TMSi reaction products was performed with a quadrupole mass spectrometer working in the positive EI mode (70 eV). The relative frequency of the TMSi H thioacidolysis monomer was then estimated by selective monitoring of its *m/z* 239 base peak and of the int. standard (docosane) $[M]^+$ at *m/z* 310.

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