Application of Light Microscopy for Studying the Processes of Formation and Decomposition of Wood

Galina F. Antonova, Victoria V. Stasova

Abstract — For understanding the processes of wood formation or of any impact of mechanical/chemical treatments on mature wood it is important to evaluate the changes in wood structure. During the study of wood formation in *PInus sylvestris* L. and *Larix sibirica* Ltb. and the impact of acoustic waves on oak (*Quercus robur* L.) wood we used the light microscopy and such metachromatic dye as cresyl-violet. Annual wood ring formation was studied by some directions: 1. to estimate the distribution of the processes of cell wall production by cambium, the development of primary and secondary walls of early and latewood tracheids in the course the seasonal wood ring formation and the effect of external factors on morphological characteristics of tracheids; 2. to record the presence of starch granules as the index of carbon store or its expenditure in favorable and unfavorable external conditions during phloem development in pine and larch; 3. to study the biochemical changes in mono-, di- and polymeric compounds (carbohydrates, organic and phenolic acids, hemicelluloses, cellulose and lignin), included in biosynthesis and the formation of wall structure of tracheids during them development. Light microscopy was also used to check of cell wall development and lignification in pine (*Pinus sylvestris* L.) callus growing under different conditions of the cultivation to compare lignification *in vivo* and *in vitro*. The structure of oak (*Quercus robur*) wood from different habitats and its destruction under ultrasound was studied with light microscopy to estimate the influence of the site conditions on oak wood structure and the changes in that under ultrasound of various powers. The examples of light microscopy applying in these fields are given in the paper.

Keywords: annual wood ring formation in coniferous, biochemical changes in cell wall compounds, control of (*Pinus sylvestris* L.) callus growth, destruction of oak (*Quercus robur*) wood under ultrasound waves, light microscopy, metachromatic dye cresyl-violet.

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1 Introduction

During the study of wood formation or of any impact of mechanical/chemical treatments on mature wood it is important to evaluate the changes in wood structure for understanding the proceeding processes. Interrelation of the degree and stage of cell development and biochemical changes in them in the course wood formation can help to interpret physiological events in cell walls of forming tissue. The determination of the destruction degree of wood and its components can help to control and/or optimize the process. In both cases light microscopy successively records the changes in wood structure in the combination with such metachromatic dye as cresyl-violet. The staining of tissues under metachromatic dyes changes due to both the alteration in the accessibility of chemical groups in substrate and of dye molecules aggregation level. Preliminary study of the mechanism of interaction between cresyl-violet and the components of developing woody tissue showed that the sorption of the dye occurs due to carboxyl groups of wood components [1]. The change in the density of spatial structure of wood modifies the staining of the tissue under cresyl-violet. The formation and development of xylem cells, accompanying by consecutive deposition of hemicelluloses, cellulose and lignin, leads to the reorganization in spatial structure of the cell walls and the consolidation of the tissue itself especially during lignification. From other side the destruction of wood under, for example, such physical impact as ultrasound waives leads to the disintegration of wood compounds, what is reflected on the distribution of the dye in tissue and changes of its staining.

The examples of the application of light microcopy during the study of wood formation in coniferous (morphological and biochemical aspects) and of ultrasound waive effect with various powers on oak wood are presented in the paper.

2 Results of applying of light microscopy

2.1 Wood formation

2.1.1 Wood formation in Scots pine (*Pinus sylvestris* L.) and larch (*Larix sibirica* Ltb.) stems were studied by some ways. By one of them the annual wood layer growth and the influence of external factors on morphological characteristics of main structural elements of coniferous tissues (tracheids) in the course the season was estimated. It is well known that annual wood rings of pine and larch consist of early- and latewood tracheids differed by radial diameters and cell wall thickness, where accumulated principal biomass of formed xylem. To find the reason of such differences the cores were extracted from the living tree stems through certain temporal intervals in the season and fixed with formalin-acetic acid-ethanol (5:5:90). At the core cross-sections the number of cells, radial and tangential sizes of tracheids and their lumens were estimated after the staining with cresyl-violet to inspect the state of the processes of cell production by cambium, the development of primary and secondary walls of early and latewood tracheids during the season as well as the beginning of lignin deposition [2], [3], [4]. The deposition of lignin was also checked with phloroglucinol-hydrochloric acid (reaction of Wiesner). Both of the dyes showed equal staining of the beginning

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of lignification. Obtained data were used to calculate the cambial activity, the rate and the duration of the development of radial diameters and secondary walls of tracheids in the zones of growth and secondary wall thickening and the cell wall biomass increment in certain periods of the growth season. All data and the data on temperature-precipitation in the each temporal period were used to determine the optimal conditions for the development of tracheid parameters. Such approach permitted to establish different effect of external factors on radial diameter growth and secondary cell wall thickening (Tab.1) and, as the result, to understand the reasons of the discrepancies during the formation of early- and latewood tracheids under changing weather conditions in the season [3], [4]. The radial diameter of tracheids depends on the development rate in growth expansion zone whereas cell wall thickness depends on the duration in secondary wall thickening zone. The basis is the different physical causes of these events.

TABLE 1
Optimal values of temperature and precipitation for the development of tracheids during the formation of annual wood ring in *Pinus sylvestris* L. stems

Characteristics of annual wood layer	*Tmean daily (C)*	*Tmax of day (C)*	*Tmin of night (C)*	**Precipitation (mm)**
The number of cells in radial row	20	24	9	3-4 *
Radial diameter	21-23	27-28	8-9	15-20**
Cross-section area of cell walls	16	20-21	9	1,9- 2,3**

* - Precipitation in a 24 hour period,
** - Total precipitation during entire development period of tracheids in the zone

In parallel with wood formation in larch and pine stems the development of phloem layer was studied to estimate the production of phloem cells by cambium and the changes in chemical compounds, in particular, the presence of starch grains as the index of carbon store or expenditure in favorable and unfavorable external conditions [5]. It is well known that in the dependence on external factors an excess of photo-assimilates can be stored within parenchyma cells of phloem and xylem as starch grains, which then be consumed in cellular syntheses. At the beginning of annual ring wood formation the starch grains have been found to be exported from xylem ray parenchyma cells, then gradually xylem resin cannels and after that from rays and axial parenchyma of phloem (Fig.1 a, b). The last events are especially observed in July when photosynthesis is suppressed by high temperatures and the lack of substrates for synthetic and energetic processes arises.

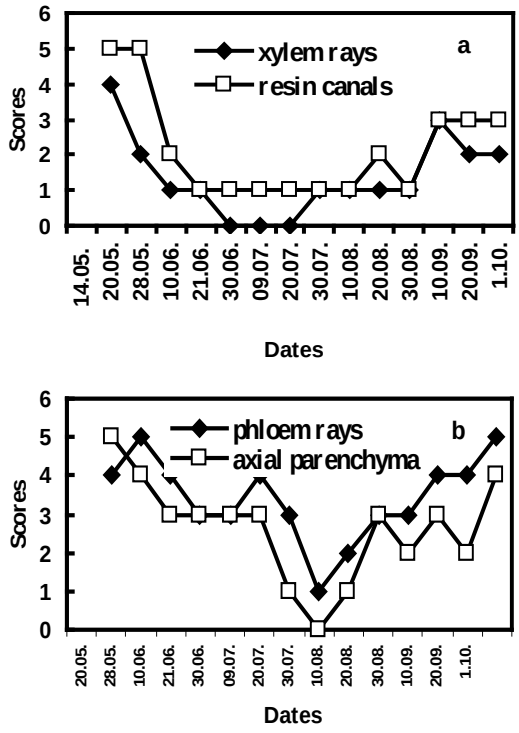

Fig. 1. The content of starch (in scores) within the cells of the rays and resin canals of xylem (a) and of rays and axial parenchyma of phloem (b).

2.1.2 To understand biochemical reasons of the differences in morphological parameters of early and late tracheids in the course of wood formation the changes in mono-, di- and polymeric compounds (carbohydrates, organic and phenolic acids, hemicelluloses, cellulose and lignin), included in biosynthesis and the formation of cell wall structure, were analyzed. With this purpose the cell layers with different developmental degree were sampled consistently layer by layer from cuttings of the living trees and the development state of the cells in the each layer was tested with cresyl-violet (Fig.2). This permitted to check thoroughly cell developmental stages during their sampling. The tissues sampled were immediately fixed with ethanol at the final concentration not exceeding 80%, weighed, and kept in refrigerator until the analyses. Careful sampling permitted to find the specific peculiarities in the deposition of pectin, arabinogalactans, the fractions of A and B hemicelluloses and cellulose at developmental stages of primary and secondary walls of the tracheids during earlywood and latewood formation in larch (*Larix sylvestris* L.) and to compare the data with morphological structure of forming cells [6]. So, nothing any limitation by substratum (carbohydrates) has been found for wall thickening of early tracheids, cell wall thickness of which considerably less than late tracheids (Fig.3). The reason of such differences is the various durations of cell development in secondary thickening zone as noted above [3], [4]. There were the differences in deposition of pectin substances, uronic, phenolic and ascorbic acids

during cell wall structure formation of early and latewood tracheids in larch [6], [8, p. 443-466]. Furthermore, the intensity of lignin deposition in both larch and pine has been found to be different in the course of the maturation of earlywood and latewood tracheids [9], [10]. It increases gradually to the last stage

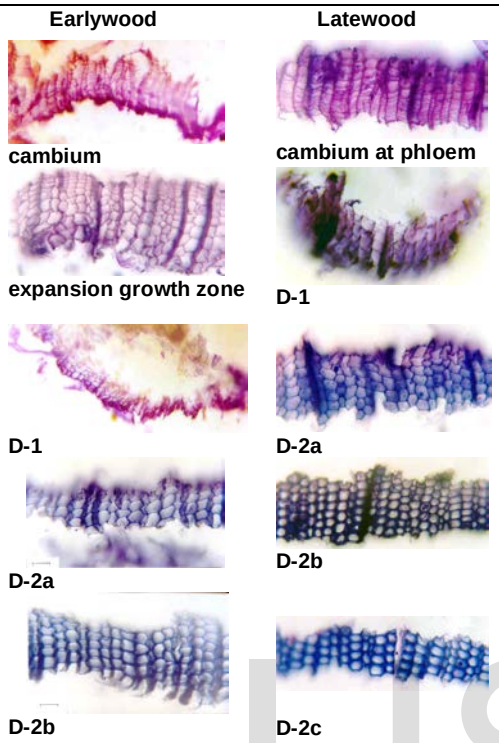

Fig. 2. The cell layers sampled from the zones of cambium, growth by expansion and secondary wall thickening before (D1) and after the beginning of lignin deposition during earlywood (D2a, D2b) and latewood (D2a, D2b, D2c) formation

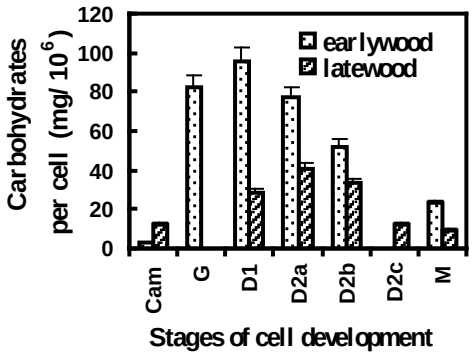

Fig. 3. Changes in the carbohydrates within the cells of cambium (Cam), growth expansion zone (G), secondary cell wall thickening zone before (D1) and after (D2a, D2b, D2c) the beginning lignification and mature xylem (M) during early- and latewood formation.

of earlywood tracheid maturation whereas it is the highest at the outset stage of lignification and declines by the end of tracheid maturation in the course of latewood development (Fig.4). This coincides with the changes in the ratio of ascorbic/dehydroascorbic acids what influences the level of oxidative reactions of lignin precursors (Fig.5). All combined data (morphological and biochemical) can serve as the base to understand the morphological differences in early- and latewood tracheids [3], [4].

Light microscopy was also used to check the changes in morphological state of callus cells and in their biochemical composition, in particular, in lignin deposited in pine (*Pinus sylvestris* L.) callus growing under different conditions of the cultivation. The study was carried out to compare lignification *in vivo* and *in vitro* [10].

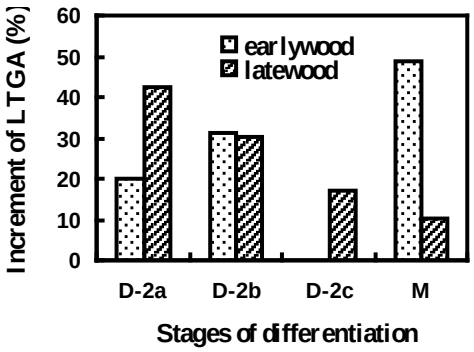

Fig. 4. The rate of the deposition lignin (LTGA) within the cells of secondary cell wall thickening zone (D2a, D2b, D2c) after the beginning lignification and mature xylem (M) during early- and latewood formation.

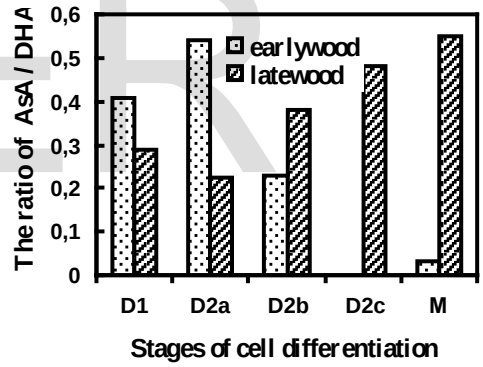

Fig. 5. Changes in the ratio of ascorbic (AsA) and dehydroascorbic (DHA) acids within the cells of secondary cell wall thickening zone before (D1) and after (D2a, D2b, D2c) the beginning lignification and mature xylem (M) during early- and latewood formation.

2.2 The impact of ultrasound waives on oak wood

Destruction of wood under a physical impact, for example, ultrasound waves was also registered by light microscopy. We studied the changes in the structure of oak (*Quercus robur* L.) wood under ultrasound in different mediums with and without pretreatment by temperature [11], [12]. The oak wood of the trees from two stands with different wet growth conditions (valley and hill sites) was used. The effect of acoustic waves on oak wood at air, water and water-alcohol solution with different expositions was investigated by the anatomical and biochemical analyses, by gel-filtration, X-ray diffraction and IR-spectroscopy The treatment of oak wood by supersonic waves, X-ray diffraction and IR-spectroscopy studies were realized in Institute of Solid state physics RAS (Russia), the morphological and biochemical

analysis were carried out in VN Sukachev Institute of Forest SB RAS (Russia). The destruction of wood was tested by the changes in the staining of oak wood cross-sections with cresyl-violet, the appearance of the splits in cell walls (Fig.6), by the variations in morphological characteristics of wood cells as well as by the changes in the chemical composition of wood and its compounds, in particularly, in lignin [11]. Specific reagents (Wiesner and

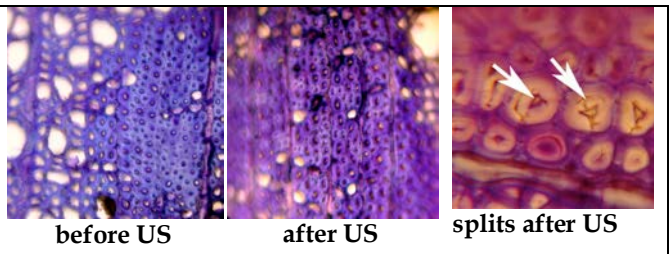

Fig. 6. Changes in the staining of oak wood and the appearance of the splits in its structure after the effect of ultrasound

Mäule) for the visualization of guaiacyl and syringyl structures in lignin showed what the latter, located within libriform fiber walls, was destroyed under ultrasound more than the former, located in middle lamella didn't practically changed (Fig.7).

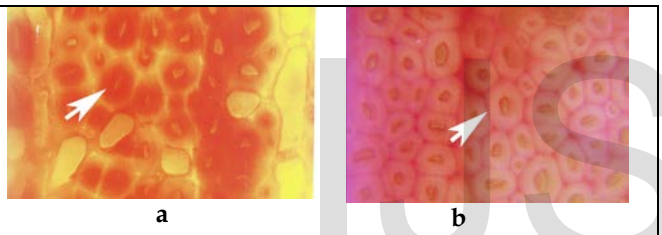

Fig. 7. Specific reactions of syringyl (a) (by Mäule) and guaiacyl (b) subunits (by Weisner) of lignin of oak wood after ultrasonic impact

Under the impact of ultrasound with frequency 19-21 kHz in a low power range at wood pieces the spatial organization of oak wood structure changed in dependence on the medium (air, 65 % water-alcohol solution and water) of the treatment and the exposition time (15, 35 min and 1 h, correspondingly) [11]. After supersonic treatment the principal changes in wood structure were observed in cell walls of libriform and its porosity. The effect of acoustic waves in air medium during 15 and 35 min on wood structure was practically similar whereas the treatment in water medium caused structural changes in middle lamella mostly. However the effect of ultrasound on morphological structure of oak wood from diverse inhabited conditions had some differences which were caused by the discrepancies in the initial structure of wood because of growth conditions.

Accordingly to biochemical analysis ultrasound treatment influenced the amounts and composition of the substances extracted by 65 % water-alcohol and water [11]. The solubility of carbohydrates and phenols increased, especially in such mediums as water and air. The treatment of wood in water-alcohol medium favored following extraction of the larger amount of free phenols. Supersonic treatment of oak wood produced the structural changes in lignin what was resulted in the increase of its alcohol-soluble fraction. This was supported by gel-filtration analysis, shown the changes in molecular weight distribution of lignin and mostly in its alcohol-insoluble fraction. According to histochemical reactions and IR spectroscopy it is syringylpropane moiety of oak wood lignin that is mainly destroyed by acoustic waves. X-ray diffractometry showed the increase in crystal part and size of elementary cell in cellulose microfibrills of oak wood treated by supersonic waves at air [11].

The raise of the power of ultrasound to 80 W/cm^2 even at short-term high power (30s) led to the increase libriform porosity and the cross-section area of small vessels, the amount of water-alcohol-soluble carbohydrates, phenolic compounds and, in particular, of free phenolic acids (Tab.2).

TABLE 2
The changes in chemical composition of oak wood under the treatment of high power ultrasound (US)

Characteristics	Valley site	Hilly site
Substances soluble in 65 % ethanol (% of dry weight)		
before US	3.18 ± 0.08	4.62 ± 0.10
after US	11.31 ± 0.12	7.21 ± 0.05
Substances soluble in water (% of dry weight) before US	7.95 ± 0.06	8.37 ± 0.08
after US	18.25 ± 0.09	14.12 ± 0.04
Lignin (% of dry weight) before US	25.6 ± 0.05	31.8 ± 0.05
after US	32.4 ± 0.06	33.5 ± 0.07
Lignin soluble in 96% ethanol (% of total amount)		
before US	10.65 ± 0.12	7.15 ± 0.07
after US	5.06 ± 0.11	4.30 ± 0.10

High energy ultrasound doesn't influences lignin localization, but effects on sub-molecular structure and the degree of solubility in alcohol. The increase in the alcohol-soluble fraction was occurred due to the destruction of lignin itself and mainly its alcohol-insoluble fraction. These changes were reflected on molecular weight distribution of lignin and its fraction studied by gel-filtration technique [12]. The data of IR-spectroscopy also supported these observations. Spectral line in the region 760-772 cm-1 becomes more uniform and the intensity of line 918 cm-1 is reduced approximately in 2 times that characterizes the instability of syringyl subunits of oak wood lignin under the impact of supersonic waves.

3 Conclusion

Thus, the application of light microscopy for the registration of morphological changes in wood structure in the combination with biochemical and physical analyses gives very valuable information during the study of the processes of wood formation in dependence on different external factors and the decomposition of a wood under physical impact.

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