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PRUNING INFLUENCE ON CHEMICAL COMPOSITION OF SPRUCE WOOD (*Picea abies* (L.) Karst.)

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Abstract. The aim of the work was to investigate the influence of pruning on wood composition in different morphological parts of the Norway spruce (*Picea abies* (L.) Karst.) trees, including heartwood and sapwood compared to normal trees as a reference. Pruning intensity during that treatment was 35–41 % of the total length of the crown for the trees with the average and less than average diameter at breast height and 28 % for the trees with diameter at breast height higher than the average. Components composition of acetone soluble extractives was analysed by gas chromatography with long and short columns, and their molar mass distribution was analysed by gel permeation chromatography. The following was determined in the pre-extracted wood tissues: cellulose (acid hydrolysis – gas chromatography); lignin (Acetyl Bromide method) and hemicellulose and pectins composition (acid methanolysis – gas chromatography). Acetone-soluble wood extractives are a complex mixture of different groups of organic compounds, i.e. both lipophilic and polar substances. The high-performance gel permeation chromatography analysis revealed the dominance of triglycerides, steryl esters, fatty and resin acids in the obtained extracts. The amount of monosaccharides in the extracts was also substantial. The chemical composition of wood tissues of the reference and pruned trees was almost similar. Compared to the reference trees, the wood samples of the pruned trees showed increased amounts of resin acids in sapwood, high amounts of lignin and double amounts of fructose in both sapwood and heartwood. The analysis results can be explained by response of trees to their pruning as a defense mechanism against plant pathogens and must be a result of the current physiological activity of those trees, possibly related to differences in crown sizes.
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Keywords: pruning, *Picea abies*, chemical composition of spruce wood, extractives, cellulose, lignin, hemicelluloses, GC, GPC.

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Introduction

Increasing forest productivity and improving wood quality is one of the priorities in silviculture. It is obvious, that the intensification of silviculture is essential to increase forestry profitability.

One of the main criteria of successful operation of timber industry segments (timber, furniture, pulp and paper production, etc.) is the quality of wood raw material. Such indicators as knot presence, wood density, and fiber length affect the quality of the final product and its production cost.

The most effective way to improve the quality of grown wood is pruning, which is not widely spread in Russian silviculture nowadays. If branches are removed in due time and this procedure is carried out in conjunction with thinning and fertilization (as a rule, at the stage of commercial thinnings), the amount of high-quality knot-free wood increases resulting in higher volumes of the best varieties of sawn timber and merchantable assortments (e.g. resonant, aviation, etc.) as well as plywood veneer as a result of peeling [1].

Pruning increases trunk quality; improves the aesthetic appearance of stands and individual trees in landscape design; decreases the class of fire danger for stands; increases the resistance of stands to windfalls, snowstorms, entomo- and phyto-pests. It was found out that the cost of trimming branches on growing spruce trees and cutting off branches during logging is practically the same [3]. Thus, it means that this operation should be carried out in an earlier period (40–50 yrs prior to the final felling).

This method of improving the quality of produced wood is known from ancient times [2, 5]. In the history of Russian forestry, the first experiments on the cultivation of trees with valuable timber were carried out in the middle of the 18th century on the territory, which currently belongs to the Republics of Chuvashia, Mari El and Tatarstan. The work was carried out in oak forests (stands designed for producing timber for shipbuilding) under the guidance of foremen M. Zelcher and I. Valentine (students of the famous forester F.G. Fokel). Over 100,000 trees were treated [2]. This silvicultural technique did not become widely used in Russia and was conducted only for experimental purposes.

Currently pruning is considered to be obligatory in silvicultural treatment as it increases the profitability of capital investments [7] and it is used in highly-developed countries [6, 12, 16, 19, 22, 23].

In Finland, for example, this operation is carried out annually in the area of 2,000–4,000 ha and its estimated cost is about two mln euros. Between 1983 and 2013 166,400 ha were treated by pruning [15]. It is known that the tree crown of coniferous species is the physiological center that controls the process of wood formation inside the trunk [4]. Therefore, controlling the crown parameters by removing the lower dry and live branches without leading to depression in the growth of the tree makes it possible to grow wood with a homogeneous, knot-free structure, which increases its acoustic, physical and mechanical properties.

However, we still do not know how deep changes in the physiology of wood formation from the chemical point of view are, i.e. what the chemical composition of coniferous species after pruning is.

The aim of the work was to investigate the influence of pruning on spruce wood composition in different morphological parts of the tree, including heartwood and sapwood compared to normal trees as a reference.

Materials and methods

Origin of the research object. The studies were carried out in the stands of Norway spruce (*Picea abies* (L.) Karst.), created in 1956 in the Taitsky forest sub-district of the Gatchina forest district (compart. 28, allot. 2) of the Leningrad Region, Russia. These experimental stands were planted on the territory, where the original trees were harvested in 1940, and then it was naturally afforested by low-value deciduous species (mainly aspen and partly alder, and birch) until the experimental plot was founded there in 1956; the canopy density at the area is 0.6. The stands are located on flat terrain with loamy and moist soil. The ground cover: *Aegopodium podagraria*, *Geranium pratense*, *Convallaria majalis*, *Oxalis acetosella*. Forest type – *Picea abies-Oxalis acetosella*, site index/bonitet – I-Ia.

In 1985 the laboratory of forest plantations of the Saint-Petersburg Research Institute of Forestry (SPbNIILKh) established permanent experimental plots (PEP) No. 157, 158, 159, and 160 (reference plot) in order to test the technology of growing high-quality wood with different density of stands. The trees of all the growth classes were single-pruned up to the height of 7.5 m.

Pruning intensity during that treatment was 35–41 % of the total length of the crown for the trees with the average and less than the average diameter at breast height (DBH) and 28 % for the trees with DBH higher than the average. The branches were removed with handsaws (up to the height of 2.0 m) and with a device, designed by LenNIIILH (previous name of SPbNIILKh). The pruning period was from July to October 1985. The cuts were made without damaging the bark, flush with the surface of the trunk. The stand enumeration at all the PEPs was carried out every five years, starting with the 29-year-old ones.

At present, these permanent plots are highly productive plantations of considerable scientific value (fig. 1).

Fig. 1. Trees with their branches pruned up to 7.5 m, the Taitsky forest sub-district of the Gatchina forest district, compart. 28, 2016

Characteristics of the research object. The trees of a large-size category were analysed in this study: six trees with pruned branches and six reference trees.

Preparation of wood samples for analysis. The wood samples were the kerns taken with an age borer from each tree at the altitude of 1.3 m from the soil level. They were immediately frozen and stored in the dark in hermetically sealed plastic bags at the temperature of –24 °C. Immediately before the analysis, the samples were unfrozen, then the heartwood and the sapwood were visually marked (fig. 2).

a *b*

Fig. 2. Kerns from the reference (*a*) and pruned (*b*) spruce trees

The sapwood was separated from the bark and the heartwood in the tangential direction with a knife and divided into 2–3 mm thick wood chips. They were dried in a freeze-dryer and ground with an MF 10 Basic mill (IKA-WERKE, GMBH & Co. KG, D-79219 Staufen, Germany) equipped with a 1-mm sieve.

Isolation of the extractives. Freeze-dried and milled wood was extracted with an Accelerated Solvent Extractor 200 (ASE200, Dionix, USA). 5 g of the wood sample was placed in a 33-mL stainless steel ASE-cell and extracted with acetone:water (95:5 v/v) in a nitrogen atmosphere at the temperature of 100 °C and the pressure of 2,000 Psi during 10 min (5 min×2 times). The extracts were quantitatively transferred in 50 mL measuring flasks and these volumes were adjusted with acetone.

Gravimetric analysis of the extractives. 20 mL of the extract solution was transferred into a pre-weight test tube and evaporated with a nitrogen flow on a water bath at the temperature of 40 °C. The residue was finally dried in a vacuum-desiccator for 1 h and weighed.

GC-analysis of the extractives. 0.5 mL of the extract was transferred in to a 10 mL test tube equipped with a hermetically sealing Teflon-coated screw cap; 4 mL of internal standards in methyl-*tert*-butyl ether (MTBE) solution, containing exactly 0.02 mg/mL of each: heneicosanoic acid, cholesteryl heptadecanoate, 1,3-dipalmitoyl-2-oleyglycerol (Sigma Chemical Co., St. Louise MO, USA) and betulinol (isolated and purified in the Laboratory of Wood and Paper Chemistry at Åbo Akademi University, Finland) was added; the tube content was evaporated with a nitrogen flow in a water bath at 40 °C. After additional drying in a vacuum-desiccator at 40 °C for 30 min, the extractives were silylated with 200 μL of BSFTA:TMCS:Pyridine (4:1:1 v/v/v) at room temperature overnight in the dark.

The extractives were analysed by a Gas Chromatography (GC) method using short and long capillary columns. The group analysis of high-boiling steryl esters and triglycerides was performed with the short column. The long column was used to determine the component composition of fatty and resin acids as well as the other relatively low-molar-mass compounds.

The GC-analysis on the short column was performed with a GC-instrument Perkin Elmer Clarus 500 equipped with a capillary column HP-1 (7 m×0.53 mm, film thickness 0.15 μm). The protocol for the column oven was as follows: starting temperature 100 °C, hold time 0.5 min, temperature increase rate 12 °C/min, end-temperature 340 °C, hold time 5 min. The injector was a programmable evaporator with the protocol: starting temperature 80 °C, hold time 0.1 min, temperature increase rate 50 °C/min to 110 °C, then with the rate of 15 °C/min, end-temperature 330 °C, hold time 7 min. Hydrogen with the flow rate of 7 mL/min was used as a carrier gas. The GC-instrument was equipped with a Flame-Ionization Detector (FID) heated at 350 °C. The sample volume was 3 μL (direct injection into the column).

The GC-analysis on the long column was performed with a GC-instrument Perkin Elmer Auto SystemXL equipped with capillary columns: channel A - HP-1 (25 m×0.2 mm, film thickness 0.11 μm); channel B - HP-5 (25 m×0.2 mm, film thickness 0.11 μm). The protocol for the column oven was as follows: starting temperature 120 °C, hold time 1 min, temperature increase rate 6 °C/min, end-temperature 320 °C, hold time 15 min. The injector was a programmable evaporator with the protocol: starting temperature 160 °C, temperature increase rate 8 °C/min end-temperature 260 °C, hold time 15 min. Hydrogen with the flow rate of 0.8 mL/min (20 mL/min including split) was used as a carrier gas. The GC-instrument was equipped with a Flame-Ionization Detector (FID) heated at 310 °C. The sample volume was 3 μL (split 1:24).

Gel Permeation Chromatography (GPC) of the extractives. The molar mass distribution of the extractives was analysed by an HP-GPC (Shimadzu chromatographic system; LC-10ATVp, SIL-20AHT, CTO-10ACvp equipped with two Jordi Gel DVB 500A columns (300 mm×7.8 mm) and a similar guard column (50 mm×7.8 mm), and a SEDERE SEDEX 85 LT evaporative light scattering detector (ELSD). The sample concentration was adjusted to ca 1 mg/mL of the extractives (based on the above-mentioned gravimetric determination). Tetrahydrofuran (THF) with 1 % acetic acid was used as eluent with the flow rate of 0.8 mL/min. The injection volume was 50 μL.

GC-analysis of mono-/disaccharides. Mono-/disaccharides in the extracts were determined with GC after the direct silylation of freeze-dried aliquots. Dry sample silylation was performed with a mixture of BSTFA:TMCS:pyridine (4:1:1 v/v/v) at ambient conditions overnight. The silylated samples were then analysed by a GC-FID using a PerkinElmer Clarus 500 gas-chromatograph equipped with PH-1 (25 m×0.2 mm, 0.11 μm film thickness) and PH-5 (25 m× ×0.2 mm, 0.11 μm film thickness) capillary columns, with the oven temperature programming: starting temperature 100 °C, hold time 8 min, temperature increase rate 2 °C/min to 170 °C, then with the rate of 12 °C/min, end-temperature 310 °C, hold time 7 min. The temperature of the injector and the detector was 250 °C and 320 °C, respectively. The sample injection volume was 1 μl and the split ratio 1:30.

Mono- and disaccharides were quantified against xylitol using correction factors, which were determined by a separate analysis of authentic mono-/disaccharides.

Analysis of hemicelluloses and pectins. To characterize the chemical composition of hemicelluloses and pectins in the wood samples acid methanolysis and GC were applied [26].

3 mg of the pre-extracted with acetone:water (95:5 v/v) and dried wood sample were weighed (±0.01 mg) in a 10 mL pear-shaped flask, equipped with a hermetically sealing Teflon-coated screw cap. 2 mL of 2M HCl in dry methanol were added to the flask, which was then hermetically screwed and heated in an oven at 105 °C for 5 h. During the methanolysis, the flask was carefully shaken 5 times avoiding wood particles on its wall. After the methanolysis, the flask was cooled down in the air, opened and the residual acid was neutralized with 200 μL of pyridine. 1.5 mL of the internal standard (0.1 mg/mL of sorbitol in methanol) was added to neutralize the solution and the solvent was evaporated with a nitrogen flow at 40 °C. The content was additionally dried in a vacuum-desiccator at 40 °C for 40 min and silylated with 200 μL of HMDS + 100 μL of THMS + 150 μL of pyridine overnight.

The TMS-derivatives of sugars and uronic acids were analysed with the GC method using a Varian 3400 instrument equipped with a capillary columns HP-1 (25 m×0.20 mm; film thickness 0.11 μm) and HP-5 (25 m×0.20 mm; film thickness 0.11 μm). The protocol for the column oven was as follows: starting temperature 100 °C, temperature increase rate 4 °C/min to reach the temperature of 175 °C, then the temperature rate was changed to 12 °C/min, end-temperature 290 °C, hold time 5 min. Hydrogen with a flow rate of 1 mL/min was used as a carrier gas. The injector was a conventional evaporator heated at the temperature of 260 °C. The GC-instrument was equipped with a Flame-Ionization Detector (FID) heated at 290 °C. The sample volume was 0.8 μL (split 1:20).

Analysis of cellulose. Cellulose content in the wood samples was analysed by acid hydrolysis combined with GC [26].

10 mg (±0.01 mg) of the pre-extracted with acetone:water (95:5 v/v) and dried wood sample were weighed in a 10 mL test tube equipped with a Teflon coated screw cap. Another test tube with 5 mg (±0.01 mg) of dried pure cotton linters was used to provide the overall calibration of the method. After adding a small glass ball to each test tube, both of them were placed in to the ice bath. 0.2 mL of 72 % H_2SO_4 was added to the cooled test tube with the sample, visually ensured that the wood sample was wetted with the acid; the tube was kept at the ambient temperature for 2 h. During that 2 h of the impregnation stage, the air from the wood pores was 4–5 times evacuated in a vacuum-desiccator and the testing tube was thoroughly shaken with a laboratory shaker. 0.5 mL of deionized water was added to the test tube and the content was shaken every 20 min for 4 h at the ambient temperature; then 6 mL of deionized water was added and the reaction content was kept overnight.

Hydrolysis of the wood sample was performed under pressure in the autoclave at 125 °C for 90 min (CertoClav Stem-sterilizer CV-EL 10 l/12 l, Sterilizer GmbH, Traun, Austria). When the test tube was cooled down, a few droplets of 0.04 % bromcresol green was added into the hydrolysate, then it was neutralized with $BaCO_3$ (the indicator turned colour from yellow to blue), 1 mL of 5 mg/mL of sorbitol in water (internal GC standard) was added and thoroughly mixed. The suspension in

the test tube was centrifuged for 60 min at 1500 rpm. 1 mL aliquot of supernatant was pipetted into an empty test tube equipped with a Teflon coated screw cap; the neutralized hydrolysate was evaporated with a nitrogen flow at 40 °C (2 portions of 2 mL of acetone was added to assist water removal). Finally, the hydrolysate sample was dried in a vacuum-desiccator at 40 °C for 40 min and silylated with 100 μL pyridine + 170 μL HMDS + 75 μL TMCS overnight at room temperature.

TMS-derivatives of sugars were analysed with a Varian 3400 gas chromatograph using the conditions for hemicelluloses and pectins as described above.

The cellulose content in the sample was calculated based on the amount of glucose obtained by acid hydrolysis minus glucose obtained from hemicelluloses, i.e., glucose in galactoglucomannan determined by acid methanolysis as described above, and minus glucose in mono-/disaccharides (monomeric glucose and glucose unit in sucrose).

Analysis of lignin. Lignin in the wood sample was determined by the Acetyl Bromide (AcBr) method [18]: 4 mg of pre-extracted and dried wood sample were weighed in a 10 mL test tube equipped with a Teflon coated screw cap and added 5 mL of 25 % AcBr in glacial acetic acid and 0.2 mL of perchloric acid. The tube content was heated at 70 °C for 30 min until total wood dissolution. The test tube was then cool down to room temperature, its content was transferred to 50mL measuring flacks containing 10mL 2M NaOH + 12 mL glacial acetic acid. After adjusting the solution with acetic acid to desired 50 mL volume level its UV-absorption at 280 nm was measured using a Perkin-Elmer Lambda 40 UV-VIS spectrometer (Perkin Elmer, Inc., Norwalk, Connecticut, USA). For UV-absorption measurement was used calibration with spruce milled wood lignin (MWL).

Results and discussion

Dynamics of wood growth and formation. The long-term stationary research resulted in developing a new technology of growing high-quality timber of Norway spruce [1]. This technology includes the selection of stands and trees, promising for further cultivation; the determination of the optimal height and diameter of the treated trees, as well as their age for one-, two-, and three-stage pruning; the age limit suitable for the treatment; the number of removed and left whorls (intensity of pruning); the season of the treatment; the number of trees to be treated per ha; the required tools, the methodology of using the tools, etc.

Based on the growth of the 80-year-old model spruce trees (site index/bonitet – Ia), the potential increase in the volume of high-quality knot-free wood per ha was calculated. The amount of this wood will be 150–180 m^3/ha by the harvesting age [1]. Since this type of wood is considerably more expensive (by 4–10 times according to different sources) than the non-treated, pruning should be considered as a perspective investment.

Extractives. The total amount of acetone-soluble extractives (gravimetric analysis) was found to be higher in sapwood than in heartwood, i.e. 15.4–18.1 mg/g wood versus 12.9–11.1 mg/g wood, respectively for the reference and pruned trees (table 1). The abundance of extractives in sapwood is well established and, probably related to living parenchyma cells, which serve as the foundation of the ground tissue system in trees.

Table 1

Extractives isolated from the sapwood and heartwood from the pruned and reference spruce trees, µg/g wood

Component	Sapwood		Heartwood	
	Reference	Pruned	Reference	Pruned
Total extractives (gravimetric analysis)	15,400	18,100	12,900	11,100
Fatty acids				
14:0	12.2	10.6	2.8	3.2
16:1	12.6	13.5	8.9	7.0
16:0	87.4	101.3	30.8	33.3
17:0	31.1	37.2	28.5	16.3
18:3	48.2	46.5	157.2	150.8
18:2	81.0	97.9	252.5	237.6
18:1(9)	97.0	131.1	96.4	75.9
18:1(11)	21.9	28.3	28.4	24.3
18:0	26.9	34.2	18.2	16.9
20:3	12.0	15.4	29.2	27.5
20:0	17.7	23.5	6.9	9.7
22:0	17.8	17.2	39.6	32.3
24:0	21.1	16.3	52.1	38.0
Sum	486.9	573.1	751.4	673.0
Resin acids				
Pimaric acid	52.2	62.2	40.3	31.1
Sandaracopimaric acid	108.6	140.1	81.8	68.7
Isopimaric acid	185.4	231.5	147.4	117.4
Palustric acid	26.2	54.5	51.9	37.0
Levopimaric acid	2.3	4.2	6.0	6.1
Dehydroabietic acid	701.3	917.9	527.3	439.9
Abietic acid	45.9	55.5	78.4	57.7
Neoabietic acid	5.8	12.5	16.1	11.9
Sum	1,127.6	1,478.3	949.2	769.6
Saturated fatty alcohols				
Docosanol	51.2	57.0	56.6	53.1
Tetracosanol	40.5	36.3	80.3	65.1
Sum	91.7	93.4	137.0	118.2
Lignans				
Todolactol(1)	2.7	2.9	4.0	2.6
Todolactol(2)	42.4	16.2	76.9	72.2
Isolariciresinol	3.9	4.3	4.0	2.6

Table 1 ending

Component	Sapwood		Heartwood	
	Reference	Pruned	Reference	Pruned
Lignans				
Secoisolariciresinol	9.8	5.3	30.1	28.9
Conidendric acid	5.4	2.5	19.3	16.1
Matairesinol	9.0	4.8	28.9	21.1
Lignan A	4.9	8.8	9.0	8.5
HMR(1)	15.0	6.6	105.9	77.7
HMR(2)	103.5	35.2	354.7	234.1
Conidendrin	12.1	7.3	16.4	21.1
Pinoresinol	7.2	5.2	18.9	18.4
Sum	215.9	99.2	668.1	503.2
Sterols				
Campesterol	38.7	36.7	70.7	59.5
Campestanol	7.1	8.4	10.2	9.3
Sitosterol	93.1	77.3	219.9	175.9
Sitostanol	33.9	34.0	42.6	37.7
Sum	172.8	156.4	343.4	282.4
Triterpenes				
Squalene	10.1	22.7	12.2	11.8
Lupeol	4.9	3.7	2.7	1.6
Cycloartenol	3.2	3.2	5.8	6.3
Methylcycloartanol	4.8	5.3	7.3	7.5
Sum	23.0	34.9	28.2	27.2
Steryl esters and Triglycerides				
Steryl esters	373.4	477.9	1021.6	918.0
Triglycerides	551.5	616.5	489.7	351.8
Mono-/di-saccharides				
Galactose	65.1	90.8	13.7	9.1
Glucose	160.8	172.9	24.9	106.3
Fructose	1,055.4	2,093.6	75.0	136.8
Saccharose	24.2	14.7	15.1	19.1
Sum	1,305.4	2,372.0	128.8	271.3
Total extractives (GC-analysis)	4,348.2	5,901.8	4,517.3	3,914.7

The acetone-soluble wood extractives are very complex mixtures of different groups of organic compounds and mainly consist of both lipophilic and polar substances (fig. 3, table 1). The GPC analysis revealed the dominance of triglycerides, steryl esters, fatty and resin acids in the obtained extracts. The amount of monosaccharides in the extracts was also substantial.

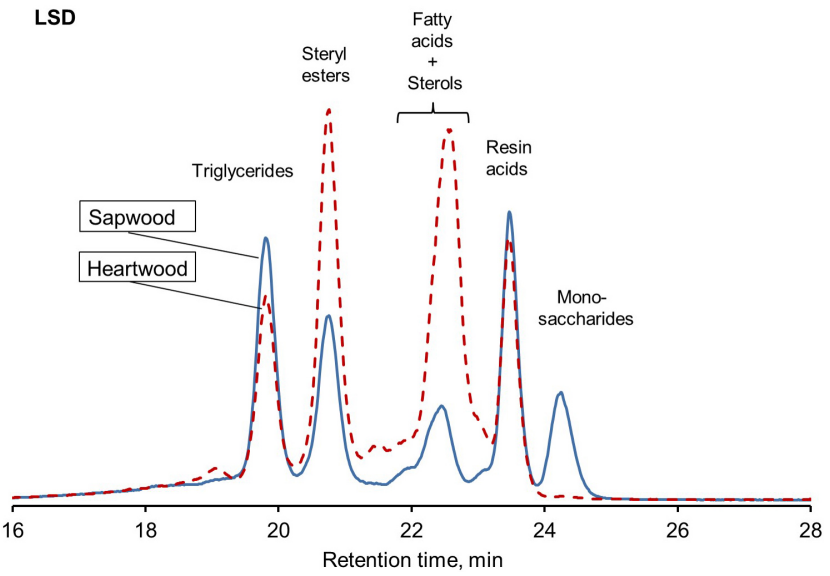

Fig. 3. GPC chromatograms of the extractives from the sapwood and heartwood of the pruned spruce tree

It should be noted, however, that monosaccharides are almost not soluble in THF (eluent in the GPC analysis), so the real amount of them in the acetone-soluble extractives should be much higher than shown in the GPC chromatogram.

The differences between group compositions of the extractives from the sapwood and heartwood were mostly quantitative. The sapwood extractives contain a bit more triglycerides, resin acids, and monosaccharides (THF-soluble part). On the contrary, the heartwood extractives included a higher quantity of steryl esters and fatty acids. The component composition of the acetone-soluble extractives from different wood tissues from the reference and pruned trees is shown in table 1.

Resin acids in sapwood were the dominating group of the extractives obtained from the pruned trees, amounting to 1,480 μg/g wood. The higher content of resin acids in sapwood from the pruned trees compared to that from the reference trees (1,130 μg/g wood) was mostly due to the higher content of dehydroabietic acid (920 μg/g wood) as well as isopimaric (230 μg/g wood) and sandaracopimaric (140 μg/g wood) acids. Such differences in resin acid content in sapwood between the reference and pruned trees can be explained by the defense mechanism of wounded trees against plant pathogens, induced by pruning [11, 13, 17].

The amount of resin acids in heartwood was also high, but lower than that in sapwood, i.e. 950 μg/g wood and 770 μg/g wood in the reference and pruned trees, respectively. The most abundant resin acid in heartwood was dehydroabietic (530 μg/g wood and 440 μg/g wood in the reference and pruned trees, respectively).

Steryl esters in heartwood comprised the second most abundant group of the extractives of both reference and pruned trees, i.e. 1,020 μg/g wood and 920 μg/g wood, respectively. This was several times higher than the steryl esters content in the corresponding sapwood.

Fatty acids represented the third abundant group of the extractives. Their content in the heartwood of both reference and pruned trees was slightly higher than that in the sapwood, i.e. 750–670 μg/g wood versus 490–570 μg/g wood, respectively. The individual compounds dominating in this group were unsaturated acids C18:2, C18:3, and C18:1(9), which together comprised more than 65 % of the fatty acids from all heartwood samples. In sapwood, the most prominent fatty acids were C16, C18:2, and C18:1(9), which covered more than 50 % of the fatty acids in all sapwood samples.

Triglycerides amount in the extractives was similar to that of the fatty acid group. More triglycerides were found in the sapwood of both reference and pruned trees in comparison with the heartwood, i.e. 550–620 μg/g wood versus 490–350 μg/g wood, respectively. This difference in triglycerides distribution between sapwood and heartwood is typical for a healthy spruce tree and well documented in the literature [20, 29].

Lignans content in heartwood was substantial and several times higher compared to sapwood. Their content in the reference trees was higher than that in the pruned trees, i.e. for sapwood it was 220 μg/g wood versus 100 μg/g wood, respectively; for heartwood – 670 μg/g wood versus 500 μg/g wood, respectively. High lignan content in heartwood compared to sapwood is in good agreement with the literature data [29]. Hydroxymatairesinol isomers HMR(1) and HMR(2) were the most prominent in this group of extractives from all the trees and amounted to more than 40 and 60 % of total lignans in sapwood and heartwood, respectively.

Sterols, a typical group of extractives found in spruce wood; their distribution pattern in a tree stem was similar to steryl esters, i.e. more sterols were found in heartwood than in sapwood: 340–280 μg/g wood versus 170–160 μg/g wood in the reference and pruned trees, respectively. Sitosterol was the most abundant compound in this group of extractives; it amounted to more than 50 % of total sterols in all the trees.

Saturated fatty alcohols were represented in the extracts by two compounds, i.e. docosanol and tetracosanol. Their content in heartwood was notable and a bit more prominent than that in sapwood, i.e. 140–120 μg/g wood versus 90–80 μg/g wood in the reference and pruned trees, respectively.

Triterpenes were also found in all the extracts in a small and approximately the same quantity in the range of 20–30 μg/g wood. The most prominent compound in this group of extractives was squalene, it constituted about half of their total amount.

Mono-/di-saccharides represented a large portion in the sapwood extracts from all the trees, i.e. 1,310 μg/g wood and 2,370 μg/g wood in the reference and pruned trees, respectively. In heartwood the amount of those sugars was found to be dramatically lower, i.e. 130 μg/g wood and 270 μg/g wood in the reference and pruned trees, respectively. Fructose was the most prominent monosaccharide in the sapwood (80–90 % of extracted sugars) and heartwood (50–60 % of extracted sugars) from all the trees. Such differences in sugar content from different wood tissues are well known; they reflect the biological activity of the living versus dead parts of a tree (sapwood versus heartwood). In this case, it's relevant to note, that both sapwood and heartwood contained a double amount of fructose in the pruned trees compared to the reference trees. As sugars reserves are produced in a tree by the photosynthesis in the

leaves, this could be an interesting phenomenon, probably related to trees pruning and the size of the crown [8].

Hemicelluloses and pectins are the second abundant group of plant constituents and structural compounds of a wood cell wall. They are a complex mixture of different heteropolysaccharides built up with pentose and hexose sugar units, including uronic acids (table 2).

Table 2

Composition of hemicelluloses and pectins in the sapwood and heartwood from the pruned and reference spruce trees

Component	Sapwood				Heartwood			
	Reference		Pruned		Reference		Pruned	
	mg/g wood	sugars ratio	mg/g wood	sugars ratio	mg/g wood	sugars ratio	mg/g wood	sugars ratio
Mannose	103.6	6.8	113.2	6.6	120.3	5.4	113.8	5.4
Glucose	31.3	2.1	35.2	2.0	42.8	1.9	41.5	2.0
Galactose*	15.1	1.0	17.2	1.0	22.1	1.0	21.2	1.0
Xylose	38.9	9.3	40.8	8.9	54.0	8.8	53.4	8.6
Arabinose	9.7	2.3	9.2	2.0	12.4	2.0	11.9	1.9
Glucuronic acid	0.7	0.2	0.5	0.1	1.0	0.2	0.9	0.1
4-O-Me-Glucuronic acid	4.2	1.0	4.6	1.0	6.1	1.0	6.2	1.0
Galacturonic acid	10.4	5.5	11.8	6.2	15.0	5.8	14.7	5.9
Rhamnose	1.9	1.0	1.9	1.0	2.6	1.0	2.5	1.0
Total	215.8		234.5		276.3		266.0	

*Some galactose units may also originate from galactan.

In spruce, galactoglucomannan (GGM) is the dominating hemicellulose: 150–166 mg/g wood and 185–176 mg/g wood in sapwood and heartwood, respectively. The sapwood in the pruned trees contained about 10 % more GGM than in the reference tree. On the other hand, a bit more GGM (ca 5 %) was found in the heartwood of the reference tree than from the pruned tree. It is possible, that GGM in the sapwood was less branched than in the heartwood since the ratio between mannose+glucose (main backbone sugar units) and galactose (a side chain sugar unit) was higher for sapwood (8.9/1 – 8.6/1) than for heartwood (7.3/1 – 7.4/1). However, a small amount of galactose units may also originate from galactan.

Xylan is a minor hemicellulose in spruce; it contains arabinose and uronic acids in the side chains and thus, carries the anionic charge. Xylan exhibited lower content in sapwood (54–55 mg/g wood) compared to heartwood (74–72 mg/g wood), which followed the distribution patterns for GGM [9, 25]. Differences between xylan distribution in wood tissues from the reference and pruned trees were not clear.

Pectins in spruce wood usually exist in a small (ca 1 %) quantity. They are highly charged polysaccharides due to a large amount of galacturonic acid, which is methyl esterified to a high degree in native wood [21]. Similar to GGM and xylan, pectin content in the sapwood was lower than in the heartwood from all the trees, i.e. 12.3–13.7 mg/g wood versus 17.6–17.1 mg/g wood, respectively. Based on the

ratio between galacturonic acid (main backbone sugar unit) and rhamnose (side chain sugar unit) it can be suggested, that pectins in sapwood/heartwood from the pruned trees were less branched than those in the wood tissues from the reference trees.

Lignin is a structural compound of the cell wall and the third abundant compound in wood. The content of lignin in different wood tissues from all the trees varied greatly (table 3). The results clearly indicate that heartwood contains more lignin compared to sapwood, as previously reported by [9, 14]. It appears, that in comparison to the wood from the reference trees the pruned trees were more lignified. Substantially more lignin was found in the sapwood from the pruned trees than from the reference trees, i.e. 257.3 mg/g wood and 237.9 mg/g wood, respectively. Similar lignin distribution profile was found in the heartwood: 294.2 mg/g wood versus 272.6 mg/g wood of lignin in wood from the pruned and reference trees, respectively.

Table 3

Lignin and cellulose content in the sapwood and heartwood from the pruned and reference spruce trees, mg/g wood

Component	Sapwood		Heartwood	
	Reference	Pruned	Reference	Pruned
Lignin	237.9	257.3	272.6	294.2
Cellulose	458.8	437.4	423.8	415.2

Lignin contributes not only to the mechanical strength of trees but can also serve as a barrier for pests and pathogens, playing an important role in the biodefense mechanism [10, 28]. Such regulation of lignin synthesis in wood as a defensive response to the tree damage caused by pruning seems to be a logical explanation of the high lignin content in the wood of the pruned trees compared to the reference trees.

Cellulose is the principal structural compound of the wood cell wall. Its distribution pattern between sapwood and heartwood was found to be inverse to the corresponding lignin content, i.e. more cellulose in sapwood than in heartwood (table 3). This is a well-established statement for coniferous wood species [9, 24, 27].

It was found that both sapwood and heartwood from the reference tree contained more cellulose than those from the pruned tree, i.e. 458.8 mg/g – 423.8 mg/g wood versus 437.4 mg/g – 415.2 mg/g wood, respectively. The low cellulose content in the sapwood/heartwood from the pruned tree can be due to the high amount of lignin in those wood tissues, as described above.

Conclusion

Spruce tree pruning is a well-known forestry technology resulting in high-quality knot-free wood with considerably high mechanical properties and value. The chemical composition of wood tissues of the reference and pruned trees was almost similar. However, compared to the reference trees, in the wood tissues from the pruned trees, there was found: a higher amount of resin acids in sapwood; high lignin amount in both sapwood and heartwood; the double amount of fructose in both sapwood and heartwood.

The first two phenomena can be explained by the response of trees to their pruning as a defense mechanism against plant pathogens. The last one must be a result of the current physiological activity of those trees, possibly related to differences in crown sizes.

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ВЛИЯНИЕ ОБРЕЗКИ ВЕТВЕЙ НА ХИМИЧЕСКИЙ СОСТАВ ДРЕВЕСИНЫ ЕЛИ ЕВРОПЕЙСКОЙ (*Picea abies* (L.) Karst.)

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Аннотация. Цель исследования – изучение влияния обрезки ветвей на химический состав древесины ели европейской (*Picea abies* (L.) Karst), включая ядровую древесину и заболонь. В качестве контрольной использовалась древесина деревьев, не подвергнутых обрезке. Интенсивность удаления части ветвей составляла: 35–41 % от общей длины кроны – у деревьев со средним и менее среднего диаметром на высоте груди и 28 % – у деревьев с диаметром выше среднего. Компонентный состав растворимых в ацетоне экстрактивных веществ был проанализирован посредством газовой хроматографии на длинной и короткой колонках, молярно-массовое распределение найдено с помощью гель-проникающей хроматографии. В предварительно экстрагированных образцах древесины определяли содержание целлюлозы (кислотный гидролиз – газовая хроматография), лигнина (метод ацетилбромида) и состав гемицеллюлоз и пектиновых веществ (кислотный метанолиз – газовая хроматография). Растворимые в ацетоне экстрактивные вещества древесины представляют собой сложную смесь различных групп органических соединений, включая липофильные и полярные вещества. Анализ полученных экстрактов с использованием гель-проникающей хроматографии выявил преобладание в них триглицеридов, стериловых эфиров, жирных и смоляных кислот, а также значительного количества моносахаридов. Химический состав древесных тканей контрольных деревьев и деревьев из опыта был схож. Однако по сравнению с первыми древесина вторых характеризовалась повышенным количеством смоляных кислот в заболони, высоким количеством лигнина и в два раза большим уровнем фруктозы как в заболони, так и в ядровой древесине. Такие результаты можно объяснить реакцией деревьев на обрезку ветвей и воздействием патогенов (т. е. срабатыванием защитного механизма растения). Полученные данные также являются отражением физиологической активности обследованных деревьев и, возможно, связаны с различиями в размере их крон.

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