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THE INFLUENCE OF STRESSES ON THE COMPONENT COMPOSITION AND STRUCTURE OF JUNIPER WOOD*

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The influence of abiotic stress on wood of the common juniper (*Juniperus communis L.*) grown in the subarctic zone of the Russian Federation is studied in the paper. It has been determined that a sharp reduction in annual growth and acceleration of biosynthesis processes of protective components take place already in the early stages of juniper wood desiccation. The lignin content increases from 28.3 to 35.2%. In contrast, the rate of formation of the polysaccharide part is significantly reduced. Owing to the lack of primary components, the possibility of polysaccharide degradation into carbohydrates increases. Changes in the component composition of wood lead to destruction of the thermodynamic compatibility of components of the lignin-carbohydrate matrix and formation of an unstable supramolecular structure of the wood substance of the cell wall. As a result, the wood substance formed in the stress period is characterised by lower mechanical strength compared with sound wood, as is proved by microscopic investigation. We can observe a lot of cracks and destruction of some layers of the cell wall at the splits in damaged wood, as well as coalescence of fragments of their destruction on the surface. Delamination of the secondary cell wall into separate layers (S_1, S_2, S_3) and absence of a tertiary wall – a warty layer are observed on the cross-sectional splitting of desiccated wood. The abovementioned changes in the structure of the juniper wood substance are observed even in the second year of desiccation, but they are typical only of newly formed wood substance and do not affect the heartwood.

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Keywords: juniper, abiotic stress, winter physiological drought, cell wall, wood substance.

Introduction

The vegetative organs of juniper and all other conifers are affected by human activity and use of these species in plantings in industrial cities with high levels of air pollution is, therefore, limited [3]. The vegetative system of juniper can also be damaged within its habitat. The most common adverse environmental conditions for most plants, including juniper, are drought, extreme temperatures, excess water and salts in the soil, excess or insufficient light, air pollution, etc. [1].

The proactive selective behaviour of any plant in response to a stressful environment is expressed in the plant's ability to regulate itself, to optimise its inner processes and to adapt to adverse environmental conditions throughout its life. Physiological and biochemical changes in plants are aimed at strengthening their defence system and may disrupt normal biosynthesis, composition and, consequently, affect the structure and integrity of cell membranes [1, 2].

Moisture deficit is among the most common abiotic stress factors in plants. Like any other stress, moisture deficit triggers a number of metabolic changes [2], slowing down the metabolism and causing both reversible and irreversible changes in the plant.

The purpose of this study is to explore the composition and morphology of common juniper (*Juniperus communis L.*) wood affected by abiotic stresses.

Subject and Methods of Study

The subject of this study is the common juniper, one of the most popular species of *Juniperus L.* genus, which is very stress-tolerant, disease-resistant and has a long lifespan [5].

In field studies performed in 2011–2013, we selected sample areas (61°40′ °N 31°09′ °E) where the tops of common juniper were damaged. The plants dried out to different extents, as evidenced by either partial or complete loss of needles and changed sapwood structure. This was especially evident in wood core samples collected using a Haglof CO300 borer during the preliminary analysis of age and approximate time when the plants started to dry. The characteristics of experimental plants are shown in Table 1.

Table 1

Characteristics of Common Juniper Experimental Plants

Age of sample*, years	Year when the drying appears to have started	Duration of drying, years
67±5	Healthy tree	0
47±5	2012	1
59±5	2011	2
67±5	2010	3

*Hereinafter, in Table 2, the data are provided with measurement error.

The radial growth was measured on cross cuts made close to the root collar of trunks. Annual growth rings were calculated using an MBS-10 binocular microscope (magnifying power of eyepiece – ×8, lens – ×2).

The wood composition was analysed in order to study how drying affects the composition and structural features of juniper wood; its specific morphology was explored.

Chips were obtained from pre-dried, clean-barked and basted juniper wood. The wood was cut using an LM-201 universal laboratory cutting mill with rotating knives and water cooling (to prevent thermo-mechanical modification of wood substance). Chips with an average fraction of 1–2 mm were used in the experiments.

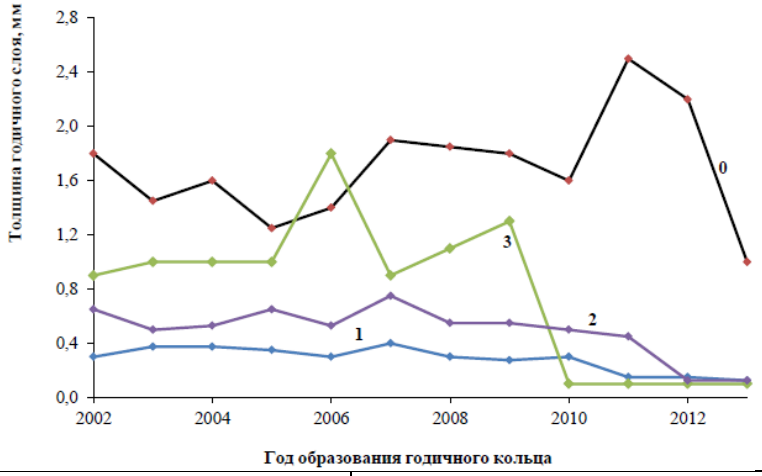
The composition of the juniper wood was determined using standard techniques [4] in four parallel measurements.

The radial and cross cuts of juniper samples were the material studied by microscopy. The splitting method was used instead of traditional cutting, which helped avoid technical difficulties in the preparation of experimental samples (pouring resin, polishing, soaking, etc.) and obtain samples with a good, lightly deformed surface. The photographs of the wood were made using a Sigma VP scanning electron microscope (Zeiss). A Q150T ES sample preparation system (Quorum) was used to coat the experimental samples with a 5 nm thick gold and palladium film to enhance contrast in the photos.

Results of Study and Discussion

When plants are drying for a long period of time, their assimilation organs partially come off. Yet, owing to transfer of nutrients from lower leaves (needles), younger upper leaves remain viable, which slows down biosynthesis and thus reduces the annual increment.

We have assessed the dynamics of annual growth of selected samples of common juniper wood in order to establish the exact time when the stress started to affect them. Fig. 1 shows changes in the thickness of the annual layer in experimental samples for the past 11 years of the juniper life cycle.



Толщина годичного слоя, мм	Thickness of annual layer, mm
Год образования годичного кольца	Year when the annual growth ring was formed

Fig. 1. Changes in the thickness of the annual layer in juniper wood samples (sample numbers 0–3 match the duration of drying (Table 1))

As can be seen in Fig. 1, all experimental juniper samples showed a decline in annual growth that overlapped in time with the estimated drying out of the trees. Results of the climatic analysis of the sampling area performed using data from the nearest Rosgidromet stations collected in the last ten years suggest that winter physiological drought is one of the most probable stress factors. For example, in 2009–2010, the period of when the mean monthly temperatures transitioned into the negatives saw little precipitation (less than 50 mm), the average temperature in December being −13.4°C, which may have caused deeper penetration of frost into the soil. The gradual increase in the daylight hours and solar intensity in the spring helped enhance transpiration and reduce the amount of moisture in plants. Consequently, in 2010, the annual increment was lower in all samples, while, for sample 3, these effects were critical and stripped the needles off the plants.

The decrease in the annual increment can be attributed to a reduced number of cells in the annual ring and their smaller diameter. Wood substance that formed under stress has lower mechanical performance compared to healthy wood.

This may be due to the changed structure of the cell membranes and is confirmed by microscopy. Photographs obtained using a Sigma VP scanning electron microscope (Zeiss) show significant changes in the integrity of the cell membranes of juniper tracheides during drying out (Fig. 2).

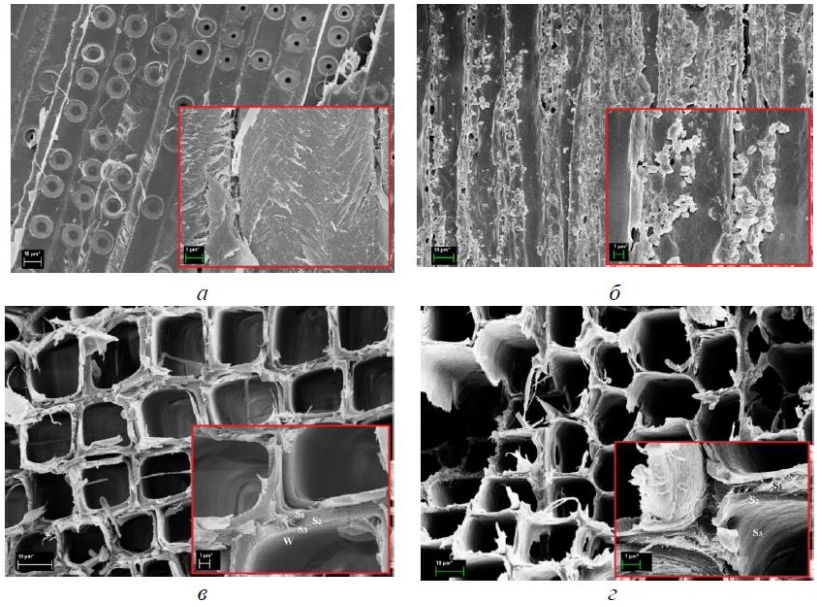


Fig. 2. Radial and longitudinal splits in healthy (a, b) juniper wood and the wood that dried out in 2012 (c, d)

а	a
б	b
в	c
г	d

As a rule, the longitudinal splits in healthy wood passes through the intercellular substance of spring tracheides (Fig. 2, *a*), as this section of wood tissue is the least solid and least resistant to splitting. In juniper wood that is drying out, longitudinal splits pass through both early (spring) and late (autumn) tracheides. This is evidenced by an almost complete absence of pores on the surface of these tracheides (Fig. 2, *b*). This is primarily characteristic of late cell formations that fulfil a mechanical function. Split directions in the autumn cell area signal decreased mechanical strength of the cell walls, which is likely to be due to significant changes in the matrix composition resulting from stress.

For this reason, even where the mechanical stress is insignificant, there are multiple cracks in tracheide cell walls in split areas, while some cell wall membranes are partly (or fully) destroyed (Fig. 2, *b*) and their destruction fragments coalesce on the surface of autumn tracheides.

Similar, even more prominent phenomena can be observed in cross cuts in the wood samples (Fig. 2, *c*, *d*). The drying of wood and changes in the composition of the wood substance cause the secondary wall to split into layers S_1, S_2, S_3, reducing the mechanical stability of the cell membranes and resulting in multiple ruptures and deformations during sampling. Most significantly, sapwood in some samples becomes so fragile that even insignificant mechanical stress may cause its destruction and dissociation.

Drying out juniper wood has no tertiary wall, i.e., the warty layer (Fig. 2, *d*) that adds durability to the cell wall from inside. The above-listed changes in the structure of juniper wood substance become apparent as early as the second year of drying, but they affect the newly formed wood tissue rather than the heartwood.

The modifications in the cell membranes are likely to be associated with changes in biosynthesis and with the proportion of the main components of the cell wall. In order to confirm these changes, we analysed the composition of the juniper wood samples.

The data in Table 2 suggest that there are significant changes in the quantitative content of the main components in juniper wood. Even at the earliest stages (alarm area), stress causes hormonal imbalance that impedes cell division and growth [1]. The major adaptation mechanisms are launched in response to primary changes, slowing down hydrolytic and catabolic reactions and enhancing the biosynthesis of protective components [8].

Table 2

Juniper Wood Composition (% of Absolutely Dry Wood) at Different Drying Stages

Duration of drying, years	Ash content	Substances extractable using			Cellulose	Lignin
		hot water	ethanol	diethyl ether		
0	0.16±0.01	2.53±0.08	4.67±0.02	3.50±0.04	44.0±0.05	28.3±0.89
1	0.37±0.01	5.33±0.03	3.93±0.07	1.80±0.07	43.4±0.12	32.9±0.04
2	0.23±0.01	2.97±0.06	4.18±0.02	3.10±0.11	42.4±0.05	34.8±0.91
3	0.20±0.02	3.48±0.04	5.19±0.01	4.40±0.05	41.5±0.29	35.2±0.20

This leads to increased biosynthesis of protective lignin as early as the first year, as is confirmed by a significant rise in the proportion of lignin components (by 4.5%). Lack of moisture and nutrients, however, leads to gradual irreversible drying of all vegetative organs and the wood, following which the growth rate becomes noticeably reduced and gradually fades out.

The biosyntheses of lignin polysaccharides and extractive substances are interconnected and competing [3, 6]. Consequently, intensified biosynthesis in one group should be accompanied by a slowdown or complete cessation of biosynthesis in the other group. Thus, drying causes the cellulose content in juniper wood substance to go down from 44.9% to 43.4% in the first year and to 41.5% in the third year. This may be attributed to a reduced rate of synthesis of this polysaccharide, as is indirectly confirmed by a reduced annual increment in the experimental samples. As known, cellulose is the main component of cell membranes and it is the intensity of its synthesis that the annual increment largely depends on. Destruction might be another reason for reduced cellulose content. These processes are mentioned by many authors, for instance, in publication [7]. If there is a lack of primary components (carbohydrates), low-molecular-weight polysaccharides in the cell wall may become depolymerised and destroyed and newly formed carbohydrates may be further involved in this process, reducing the polysaccharide content. At early stages of drying, the main part of carbohydrates produced by both photosynthesis and destruction of polysaccharides participates in the fermentative synthesis of phenylpropanoid compounds (cinnamic acid route), which leads to production of three cinnamic alcohols and some extractive substances through activated cinnamic acid derivatives. In the first year of juniper drying, aromatic extractive substances are not produced; in contrast, the proportion of components extractable using hot water, ethanol, acetone and other solvents is reduced, as is confirmed by the composition data (Table 2).

Thus, at the early stages of drying, the biosynthesis of the main components of the juniper wood matrix becomes refocused, specifically, the synthesis of lignin and protective extractive substances is accelerated. Composition changes disrupt the thermodynamic compatibility of lignin-carbohydrate matrix components and lead to formation of an unstable supramolecular structure of the wood substance in the cell wall. Changes in the structure of juniper wood substance become apparent as early as in the second year of drying, but they affect the newly formed wood tissue rather than the heartwood.

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