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MORPHOLOGICAL AND ANATOMICAL PECULIARITIES OF THE SCOTS PINE ANNUAL RINGS IN MOSS-COVERED AND LEDUM TYPES OF FORESTS

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In the introduction, it is emphasised that any plant is an indicator of the environmental conditions of its site, and it is true for every cell of a plant. The purpose of this work is to assess the possibility of differentiation of moss-covered (Pinetum polytrichosum) and ledum pine forests (Pinetum ledosum) which are characterised by quite similar site conditions on the basis of a comparative analysis of quantitative characteristics of morphological and anatomical structures of annual rings. In the main part of the article, general tendencies of the wood-ring chronology are analysed; as a result, it is determined that perennial and yearly variations for these types of forests are quite similar, which makes it impossible to differentiate between them only on the basis of a tree-ring analysis. A complex assessment of variations of individual dimensional parameters of early and late tracheids sensitive to ecological gradient impact, including the use of intentionally selected chemometric analysis algorithms, has been performed. On the basis of a discriminant analysis of projections on latent structures, an algorithm for automatic identification of a forest type has been presented, which makes it possible to receive maximum diagnostic and identification information, as well as key parameters sufficient for a classification procedure have been determined. Thus, the most substantial contribution to the differentiation between moss-covered and ledum pine forests is made by the cell wall area and late tracheid cavity area, as well as the late tracheid cell wall thickness. The classification model obtained has shown high predictive capacity; classification total mean accuracy has reached 97.48%. In the conclusion, it is stated that the established correlation between individual morphological and anatomical structures and site conditions makes it possible to use them as individualising factors when performing forensic examinations.

Keywords: forest type, Scots pine, microanatomical structure, tracheids, principal component analysis, discriminant analysis of projections onto latent structures

Introduction. Scots pine is the most widespread forest-forming tree species in the forests of the Republic of Belarus. It is represented by the Scots pine subspecies *Pinus sylvestris* L. subsp. *sylvestris L.* A soil ecotype of the named pine subspecies *P. sylvestris* L. subsp. *sylvestris* L. var. nana Pallas grows in the bogs (Molotkov, Patlaj, 1991).

Low demands on the richness and moisture of the soil determines the fact that pine forests occupy a wide edaphic area and are capable of forming closed stands in extreme conditions for other forest-forming species: on dune heights (lichen pine forests) or in raised bogs (sphagnum pine forests) (Torngern, 2018).

In modern conditions, the wide range of typological and age variability characterizes pine forests of Belarus – from monotonous monoculture plantings to preserved unique islands of old natural forests.

The widespread occurrence, the problem of preserving the biological diversity of species associated in its development with the natural dynamics of pine forests, intense anthropogenic impact secondary to a changing climate have led to a huge scientific interest in pine forests.

The reaction of Scots pine trees growing in the same conditions in the same place to changes in a complex of environmental factors is similar (Sensuła et al., 2015; Juknys et al., 2003; Dauškane, Elferts, 2011). The term «forest type» denotes the unity of forest growing conditions and communities as a unity of plants and environment. Each type of forest (type of growth conditions) differs from others in the regular variability of radial growth (Liang et al., 2019; Aldea et al., 2021). Since the differences in the amplitude of the values of the width of the annual layers can be quantified, this makes it possible to clarify the belonging of a certain plantation to one or another type of growing conditions. However, there are a series of forest types and growing conditions, within which the variability of the increment of pine stands is relatively similar in many respects, for example, pine forests growing on soils with excessive moisture (Esper et al., 2001). We believe that in such cases, the use of data on the morphological and anatomical structure of the annual layers will make it possible to count previously unused layers of information and to distinguish those that grew up in approximately the same conditions.

Therefore, the life form of plants is known to be genetically determined and determined by hereditary qualities (Greenberg, 1996; Kozik et al., 2019). However, depending on the conditions of the place of growth in nature, some variation is observed not only in the external characteristics of the same plants, but also in the signs of their anatomical structure. At the same time, individual characteristics and the narrowest group characteristics are formed precisely under the influence of environmental conditions, i.e. plants adapt their structure (Raza. et al., 2020; Anderson, Song, 2020). This adaptation occurs during the processes of cell division and differentiation (Pierre-Jerome et al., 2018).

The objective of this paper is to find out the possibility of differentiating pine forests growing in the long-moss and ledum types of forests according to the morphological and anatomical parameters of the annual layers.

Materials and methods. The study used dendrochronological material (drill cores) from 10 temporary sample plots (hereinafter referred to as TSP), laid during fieldwork in 2016–2017 on the territory of the Tomashevsky forestry of the Brest forestry enterprise; 5 for each of the studied forest types (tab. 1).

On each TSP, from 20 trees of the highest Kraft classes (dominant and co-dominant), a Haglof age drill took drill cores (2 per tree) from opposite sides perpendicular to the longitudinal axis of the tree trunk at a height of 1.3 m from the ground surface.

Subsequently, the drill cores were soaked for 10-15 minutes in hot water, and then the upper part 1-1.5 mm thick was cut with a pistol knife with a retractable trapezoidal blade in the transverse direction. Next, the cores were scanned using an Epson Perfection V19 flatbed scanner with a resolution of 1200 dpi. The calculations of the parameters of the radial gain were performed using an automated workstation «DendroExp» (with an accuracy of 0.01 mm) (Khokh, Kuzmenkov, 2017).

The preparation of slides consisted of the following stages: digestion of cores in boiling water for 15 min for softening, obtaining cross sections of the last 20 annual rings with a thickness of 20 microns and fixing the obtained sections in glycerin.

The slides were analyzed using the MCview software (LOMO-Microsystems). Dimensional characteristics were measured in 5 adjacent rows of cells in each layer by the continuous measurement method with an accuracy of ≤ 2 µm.

Average values were obtained by averaging the measurement results over the annual layers of each of the studied trees on the TSP. To distinguish between early and late tracheids, the Mork rule was used, according to which the late tracheids include cells in which the radial thickness of the double cell wall is greater than or equal to the half-width of the lumen (Butterfield, 2003).

Statistical processing was carried out using the statistical packages Statictica v.10.0 and The Unscrambler X v.10.4.1.

Results and discussion. Based on the calculated parameters of radial growth for each TSP, generalized tree-ring chronologies (hereinafter referred to as TRC) were constructed.

Table 2 shows the statistical indicators of the qualitative assessment of the available dendrochronological material (the number of the TRC corresponds to the number of the TSP).

The analysis of the obtained results showed that the width of annual layers in the studied pine trees varied from 0.13 to 2.57 mm. The value of the standard deviation is maximum for trees growing on TSP No. 2 (0.42), and the minimum for trees on TSP No. 8 (0.15). The maximum coefficient of variation is typical for TRC No. 9 (63.3%), the minimum – for TRC No. 3 (30.1%). Trees on each TSP are distinguished by high synchronicity of year-to-year variability of radial growth, which is confirmed by high inter-serial correlation coefficients: from 0.65 to 0.79 with an average value of r = 0.72.

Table 1.

Brief forestry and taxation characteristics of the objects of study

Forest type	№№ TSP	Forest quarter	Forest inventory subcompartment	Site productivity type	Stand composition	Average age, yea	Quality class	Stand density	Height (h), cm	Diameter at a height of 1.3 m (d),	h/d
Pinetum polytrichosum	1	36	8	A4	10Pn	80	II	0,6	24	33	0,7
	2	40	2	A4	9Pn1B	80	III	0,7	22	28	0,8
	3	52	11	A4	8Pn2B	90	II	0,6	20	28	0,7
	4	53	2	A4	9Pn1B	80	II	0,6	25	32	0,8
	5	57	2	A4	10Pn + B	80	II	0,5	24	36	0,7
Pinetum ledosum	6	55	3	A5	10Pn	80	IV	0,7	18	21	0,9
	7	281	10	A5	5Pn	90	IV	0,8	16	21	0,8
	8	281	11	A5	10Pn	90	IV	0,9	18	22	0,8
	9	282	6	A5	10Pn	90	IV	0,8	17	18	0,9
	10	282	7	A5	5Pn5B	90	IV	0,8	18	20	0,9

Table 2.

Statistical indicators of generalized TRC

№№TRC	M_x, mm	min, mm	max, mm	S_x	m_x	CV, %	COR	SNS	SNR
1	0,59	0,22	1,61	0,21	0,02	35,4	0,75	0,19	60
2	0,77	0,31	2,57	0,42	0,04	54,9	0,79	0,17	75
3	0,71	0,37	1,45	0,21	0,02	30,1	0,75	0,16	60
4	0,74	0,37	1,81	0,28	0,03	37,9	0,68	0,14	43
5	0,70	0,35	1,68	0,24	0,02	34,4	0,73	0,15	54
6	0,42	0,13	1,87	0,22	0,02	52,3	0,70	0,24	47
7	0,43	0,13	1,41	0,24	0,02	56,1	0,65	0,27	37
8	0,44	0,23	1,47	0,15	0,01	33,9	0,71	0,23	49
9	0,48	0,21	2,44	0,30	0,03	63,3	0,70	0,19	47
10	0,52	0,24	2,29	0,28	0,03	54,4	0,70	0,22	47

Note: M_x *– the average annual layer width; min, max – the maximum and minimum annual layer width;* S_x *– the standard deviation;* m_x *– the standard error of the mean; CV – the coefficient of variation; COR – the average intra-tree correlation; SNS – the sensitivity coefficient; SNR – the signal to noise ratio.*

Sensitivity coefficients range from 0.14 to 0.27; at the same time, their growth was established with an increase in soil moisture (SNS for the moss pine forest is 0.15; SNS for the ledum pine forest is 0.23, the differences are significant at the 1% significance level, or at $p < 0.01$).

The calculated signal-to-noise ratio (SNR) ranged from 37 to 75, i.e. generalized chronologies contain a high variability explained by the influence of climatic factors (comparison for this parameter is legitimate, since the sample size is the same for all TRCs.

Further, the structure of dendrochronological data was analyzed using the principal component analysis (PCA). The results obtained are presented in the PCA-accounts graph (Fig. 1).

According to the results of the analysis, it can be seen that the location of the projections, which are a reflection of drill cores from Scots pine from the long moss and ledum types of forest, is generally random, and therefore it is not possible to differentiate the studied forest types only on the basis of dendrochronological analysis.

Therefore, further we carried out a quantitative analysis of the morphological and anatomical parameters of the last 20 annual layers. 20 drill cores were examined from each TSP.

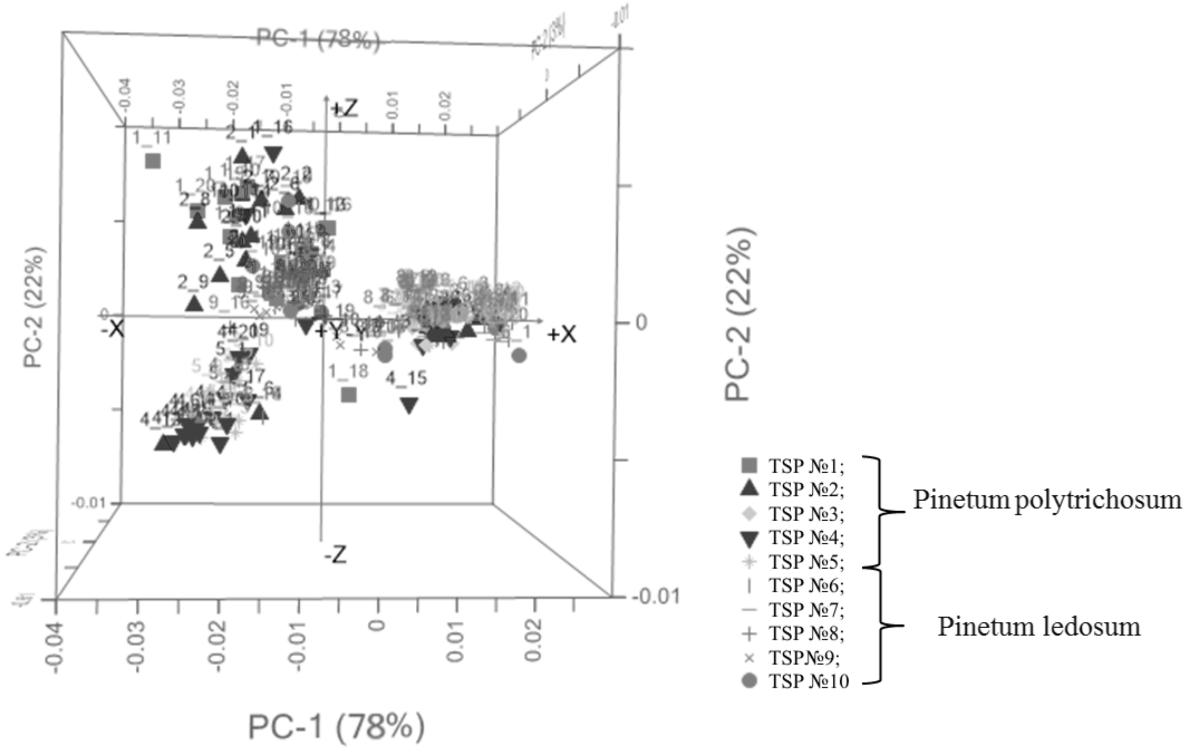

Fig. 1. Chart of PCA accounts for the studied TRC

According to the analysis of the variability in the width of annual layers, mm no differences between the ledum pine forest and the long-moss pine forest were revealed ($p > 0.05$) (Fig. 2), which indirectly confirms the fact that the general trends in tree-ring chronologies (long-term and annual fluctuations) for the data forest types are very similar. Statistically significant differences ($p < 0.001$) were revealed in the width of late wood for the studied forest types (Fig. 3).

Table 2 presents the calculated quantitative indicators of the morphological and anatomical parameters of the annual layers of Scots pine (separately for each of the studied forest types).

The results of our studies indicate that statistically significant differences were found for the following anatomical parameters of annual layers: the number of early (N_{ET}) and late tracheids (N_{LT}), radial diameter of late tracheids (R_{LT}), cell wall thickness of late tracheids (W_{LT}), the radial diameter of the cavity of the late tracheids ($L_{R\ LT}$), the area of the cell wall of the late tracheids ($S_{CW\ LT}$), as well as the area of the cavity of the early ($S_{C\ ET}$) and late tracheids ($S_{C\ LT}$); moreover, for 6 of the 8 parameters studied, the level of significance was $p < 0.001$.

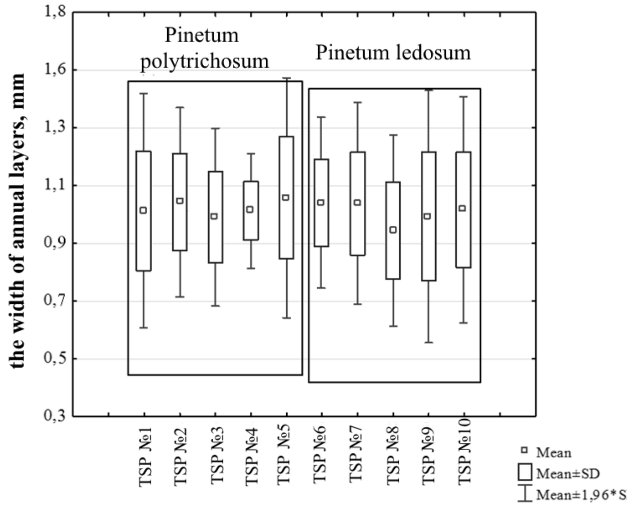

Fig. 2. Box-and-whiskers plot showing the difference in annual layer width between the studied TSP over the past 20 years

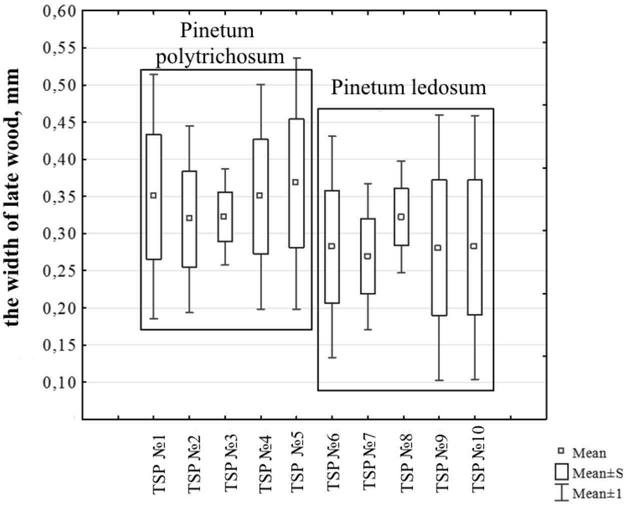

Fig. 3. Box-and-whiskers plot showing the difference in latewood width between the studied TSP over the last 20 years

Table 3.

Calculated dimensional characteristics

Measured parameters	Pinetum polytrichosum		Pinetum ledosum		t-value	p-value	Changes, %
	M	SD	M	SD			
N_{ET}, шт.	20,1	3,9	21,8	4,7	2,78	**0,006**	-8
R_{ET}, μm	34,7	6,1	33,1	5,3	2	0,047	5
W_{ET}, μm	2,5	0,5	2,6	0,5	1,41	0,159	-2
$L_{R\ ET}$, μm	31,9	5,4	31,0	6,5	1,07	0,288	3
$S_{CW\ ET}$, μm^2	277,9	33,2	270,1	42,4	1,5	0,136	3
$S_{C\ PT}$, μm^2	897,0	167,2	804,0	111,1	4,64	**0,000**	12
N_{LT}, шт.	16,3	2,1	14,7	2,0	5,52	**0,000**	11
R_{LT}, μm	22,4	2,8	20,1	2,6	6,02	**0,000**	11
W_{LT}, μm	6,7	0,5	5,0	0,8	18,02	**0,000**	34
$L_{R\ LT}$, μm	17,2	2,6	15,9	2,7	3,47	**0,001**	8
$S_{CW\ LT}$, μm^2	391,6	55,7	311,7	71,0	8,85	**0,000**	26
$S_{C\ LT}$, μm^2	121,4	31,6	99,6	19,1	6	**0,000**	22

Note: N_{ET} – the number of early tracheids in the radial row of the annual layer; R_{ET} – радиальный диаметр ранних трахеид; W_{ET} – the cell wall thickness of early tracheids; $L_{R\ ET}$ – the radial diameter of the cavity of the early tracheids; $S_{CW\ ET}$ – the area of the cell wall of the early tracheids; $S_{C\ PT}$ – the area of the cavity of the early tracheids; N_{LT} – the number of late tracheids in the radial row of the annual layer; R_{LT} – радиальный диаметр поздних трахеид; W_{LT} – the cell wall thickness of late tracheids; $L_{R\ LT}$ – the radial diameter of the cavity of the late tracheids д; $S_{CW\ LT}$ – the area of the cell wall of the late tracheids; $S_{C\ LT}$ – the area of the cavity of the late tracheids; M – the average value; SD – the standard deviation

At the same time, statistically significant differences for the radial diameter of the early tracheids (R_{ET}), the thickness of the cell wall of the early tracheids (W_{ET}), the radial diameter of the cavity of the early tracheids ($L_{R\ ET}$) and the area of the cell wall of the early tracheids ($S_{CW\ ET}$), depending on no forest type was identified.

Note that the least variability is observed in the radial diameter of the cavity of the late tracheids ($L_{R\ LT}$) (the coefficient of variation was 13%), maximum – the area of the cavity of the late tracheids ($S_{C\ LT}$) (the coefficient of variation was 24%). It is generally accepted that if the value of the coefficient of variation is less than 33%, then the data set is homogeneous, if more than 33%, then it is heterogeneous (Bedeian, Mossholder, 2000). In general, the lower the value of the coefficient of variation, the more homogeneous the population for the studied trait and the more typical is the average. It follows from this criterion that the physiological response of all 20 trees sampled on each of the TSP looks synchronized and aligned (variation coefficients < 33%).

Further, the structure of the studied morphological and anatomical parameters was also analyzed using PCA. The results obtained are presented on the PCA-accounts graph (Fig. 4).

The result of PCA of 20 TSP, presented in Figure 2, indicates the division of the studied samples into two groups, each of which fully corresponds to each of the studied forest types:

1) TSP No. 1-10 – long moss pine forest (*Pinetum polytrichosum*);
2) TSP No. 11-20 – ledum pine forest (*Pinetum ledosum*).

An exception was sample no. 6.1 with TSP No. 6 (ledum pine forest), which was removed from the dataset as an outlier in subsequent analysis.

As you can see, the projections corresponding to one type of forest do not overlap and are located in different areas of the model relative to the 2nd principal component (long moss - zone of positive values; ledum – zone of negative values) and form a single cloud. This indicates significant differences between them. It should be noted that a smaller scatter of values is characteristic of the moss pine forest.

Further, a classification model was built using discriminant analysis of projections onto latent structures (hereinafter – PLS-DA) (Lee , et al., 2018). For the analysis, the same data were used as for PCA, but all samples were preliminarily divided into classes corresponding to the studied forest types (ledum pine forest – class «1»; long moss pine forest – class «-1»). The validity of the PLS-DA model, assessed by the cross-validation method, was 97.48%.

The practical possibilities of using the classification model were tested on 20 real objects (unknown samples), from those received for forensic botanical expert research in the course of investigating cases of illegal logging.

Taking into account the calculated coefficients of significance (hereinafter – CS), the unknown samples were assigned to one of 2 classes. So, for samples No. 1-11 CS had values close to 1, which made it possible to class them as class «1» (ledum pine forest), samples No. 11-20, in turn, were characterized by negative values of CS, and therefore were assigned to the class «-1»(long moss pine forest) (Fig. 5 and 6).

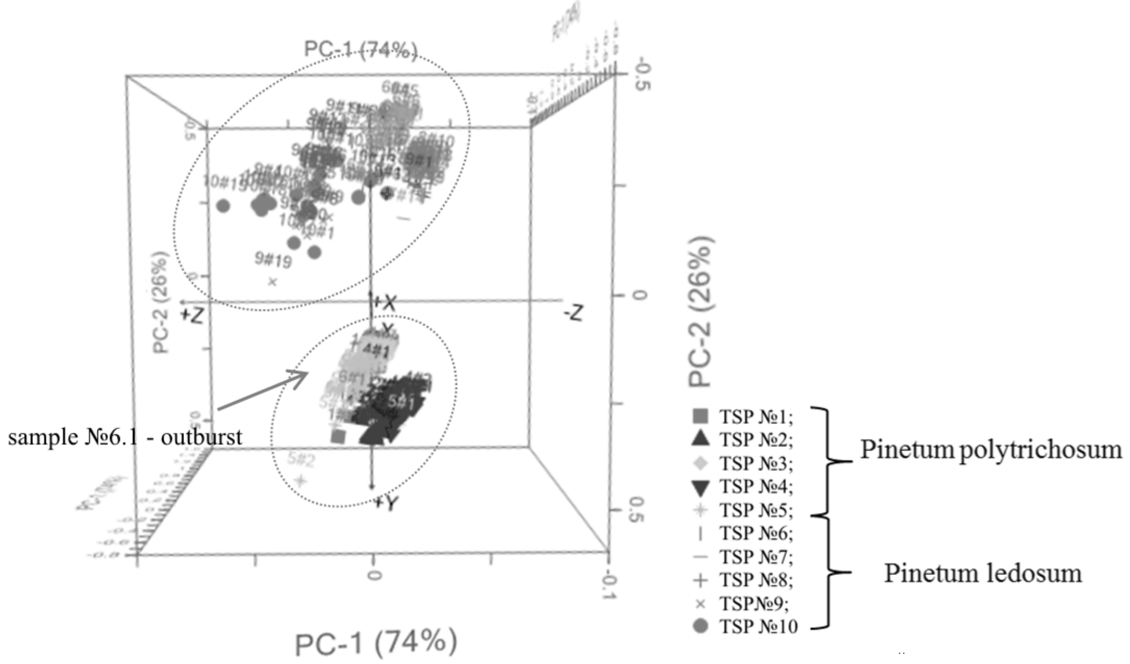

Fig. 4. Graph of PCA scores for studied morphological and anatomical parameters

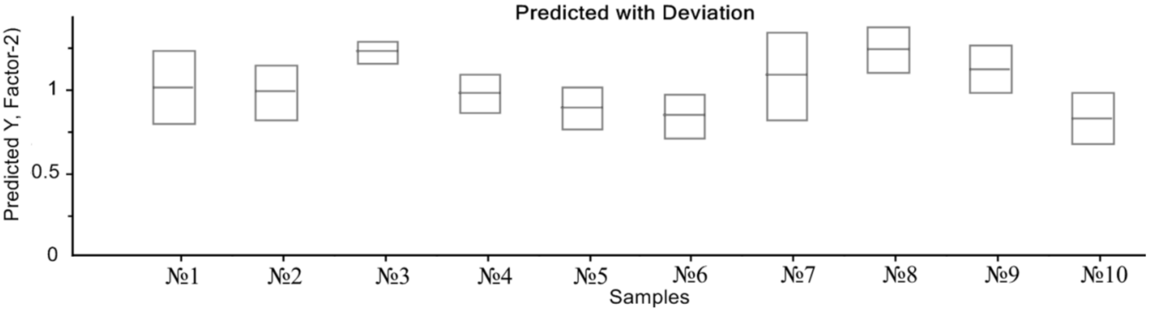

Fig. 5. Separation of unknown samples: No. 1-10 - ledum pine forest

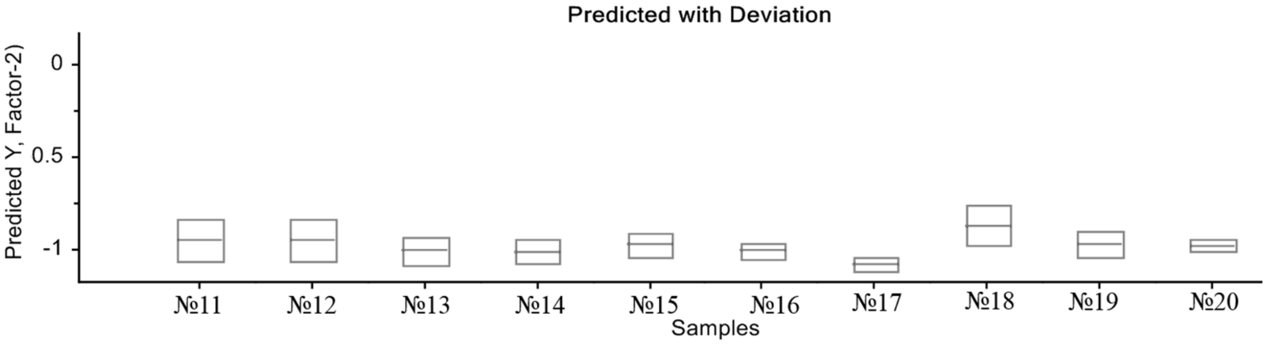

Fig. 6. Separation of unknown samples: No. 11-20 - long moss pine forest

Note that all unknown samples were classified correctly using the model we constructed.

Conclusion. As a result of the studies carried out, new experimental data were obtained and the regular variability of the morphological and anatomical structure of the annual layers of Scots pine in the moss pine forest and the ledum pine forest was revealed. The significance of individual dimensional characteristics for determining the type of forest is shown. Thus, the most informative (variable) when comparing these types of forest are the thickness of the cell wall of late tracheids (W_{LT}) – in the ledum pine forest it is on average 34% less, the area of the cell wall of late tracheids ($S_{CW\ LT}$) and the area of the cavity of late tracheids ($S_{C\ LT}$) – these dimensional characteristics for Scots pine trees are lower in the ledum pine forest by 26% and 22%, respectively. At the same time, as for the early tracheids, the differences between forest types were revealed only for 2 out of 6 morphological and anatomical parameters, while for the late tracheids all the studied parameters were significantly different. In addition, it should be noted that most of the morphological and anatomical parameters of the annual layers in the moss pine forest are higher than in the ledum pine forest (with the exception of the number of early tracheids (N) and the thickness of their cell walls (W_{ET}), which may be due to better growing conditions. In general, the results obtained suggest that the internal structure of annual layers is more sensitive to changes in environmental factors than the total width of the annual layer, and therefore its analysis can be effectively used in combination with classical dendrochronological analysis when solving issues related to the establishment of wood growing conditions (forest type).

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МОРФОЛОГО-АНАТОМІЧНІ ОСОБЛИВОСТІ РІЧНИХ КІЛЕЦЬ СОСНИ ЗВИЧАЙНОЇ В ДОВГОМОХОВИХ І БАГНОБОЛОТЯНИХ ТИПАХ ЛІСУ

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У вступі підкреслено, що будь-яка рослина є індикатором умов навколишнього середовища її місця існування. Це також справедливо і для кожної клітини рослини. Мета роботи - оцінити можливість розрізняти сосняки довгомохові (Pinetum polytrichosum) і багноболотяні (Pinetum ledosum), які характеризуються подібними умовами проростання, на підставі порівняльного аналізу кількісних характеристик морфолого-анатомічних структур річних кілець. В основній частині статті проаналізовані загальні тенденції узагальнених деревно-кільцевих хронологій; в результаті встановлено, що річні та багаторічні коливання для даних типів лісу досить схожі, що не дозволяє їх розрізнити тільки на підставі дендрохронологічного аналізу. Проведено комплексну оцінку варіацій окремих розмірних параметрів ранніх і пізніх трахеїд, чутливих до впливу екологічних градієнтів, у тому числі з впровадженням направлено обраних хемометричних алгоритмів аналізу. На основі результатів застосування дискримінантного аналізу проекцій на латентні структури запропоновано алгоритм автоматичного встановлення типу лісу, що дозволяє отримати максимум інформації діагностичного та ідентифікаційного характеру, а також визначені ключові параметри, достатні для проведення процедури класифікації. Так, найбільший внесок у відмінність між сосняками довгомоховими і багноболотяними роблять площа клітинної стінки і площа порожнини пізніх трахеїд, а також товщина клітинної стінки пізніх трахеїд. Отримана класифікаційна модель показала високу прогностичну здатність; загальна середня точність класифікації досягала 97,48%. У висновку зазначено, що встановлена залежність між окремими морфолого-анатомічними структурами та умовами проростання дозволяє використовувати їх як фактори індивідуалізації при проведенні експертних досліджень.

Ключові слова: тип лісу, сосна звичайна, мікроанатомічна структура, трахеїди, метод головних компонент, дискримінантний аналіз проекцій на латентні структури

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