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Physiological Differentiation of the Plus Trees of Scots Pine: Seasonal Status of Xylem

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Abstract: An important moment in the establishment of forestry seed orchards is the formation of their optimal composition, avoiding inbreeding depression in the outgoing seed material which occurs due to crossbreeding between closely related plus trees, the clones of which comprise the orchards. It is possible to minimize the negative effect of inbreeding by considering the hereditary aspect of the heterogeneous seed orchard material. The purpose of our work is to provide a comparative assessment of the formation and lignification of xylem in annual shoots of the plus trees of Scots pine. We investigated the formation and lignification of xylem in the annual shoots of the plus trees of Scots pine (*Pinus sylvestris* L.), found in natural forests in the Nizhny Novgorod region of the Russian Federation. Their vegetative progeny were cultivated in the clone archive stationed in the same region. Analyzing the one-type of the 1-year shoots, the time that the shoots were cut from the branches corresponded to the presence of plants in the characteristic phenological phases of seasonal development. A histochemical study of xylem was conducted, using the qualitative reactions of phloroglucinol to lignin. The power of xylem development was estimated by counting, in the radial direction, the cell rows from the core to the cambium in the microscope's field of view. A significant differentiation of plus trees was revealed in a complex of signs characterizing the level of xylem development and the degree of xylem cell lignification in the tissues of annual shoots. Phenotypic differences in the physiological state of plus trees appeared given a leveled ecological background, indicating their genotypic determinism. This was confirmed by an analysis of variance. The share of the influence of differences between the proper plus trees was between 25.16% ± 8.91% and 53.98% ± 5.48% of the total phenotypic variance of the signs of the seasonal state of xylem. Factor analysis was used to reduce the number of considered indicators of xylem physiological state. The results allowed a cluster analysis to be carried out on the basis of the normalized values of the initial xylem features as well as the principal components derived from them. The association of plus trees was done on the basis of the similarity of the multidimensional estimates of xylem seasonal condition. The objects that were the most remote from the others were identified. This information provides a reasonable approach to the formation of an assortment of Scots pine seed orchards.

Keywords: Scots pine; plus-trees; xylem; lignification; physiological condition; hereditary conditionality; factor analysis; cluster analysis

1. Introduction

The use of histochemical studies as methods for identifying the biological features and diversity of woody plants is widely accepted [1–5]. Indicators of the plant physiological state determine the adaptability of the organism to the environment, the survival of the progeny, the biological potential for reproductive activity, and regeneration ability [6–13]. The state and rate of the development of xylem in woody plants, the xylem cell formation and lignification processes, the ratio between the formation of early and late wood, as well as their hereditary conditionality are the subjects of systematic comprehensive studies [14–22]. These aspects are of particular importance in relation to native tree species, which play an important role in shaping the resource potential of forests. Among them is the Scots pine (*Pinus sylvestris* L.), which occupies a leading position in forestry not only in Russia, but also in many European countries [23–25]. In particular, information on this species is available for Germany [26], England [27], Sweden [28], Spain [29], Estonia [30,31], Finland [32], Poland [33], Belgium, and the Netherlands [34]. In studies of the polymorphism of Scots pine, the solution of the problems associated with plus selection, the establishment of seed orchards, and the increase of their effectiveness are the subjects of numerous scientific studies, conducted over long periods of time [35–40].

One of the challenges in modern forest selection is the formation of a balanced composition of the clones of plus trees on first-order seed-bearing plantations [37,41–44]. In many respects, this problem is associated with the risk of the occurrence of the inbreeding depression of the seed progeny of plus trees on seed orchards [45–51]. According to some estimates, its effect can be between 26% and 65 % [52], while other research has estimated it to be between 28.6% and 59.3% [53]. For this reason, it is important not to include closely related crosses. In coniferous plants, inbreeding depression occurs at a frequency of 10–20%, more often in seed-bearing plantations than in natural plantings, and it is recognized as the main cause of inbreeding [35,54]. According to other data [55], the self-pollination frequency of clonal seed orchards is 4%. In view of these circumstances, it is important to ensure sufficient quantitative representation of clones in seed-bearing plantations [42,56–59]. Nevertheless, it is a fact that plus selection based on mass selection according to the phenotype of the best individuals of the natural populations remains the most effective strategy for the selective improvement of forests in many countries up to the present day [60–64]. While the problem of the effectiveness of plus selection has long been discussed in the literature [65–69], unambiguous solutions or exhaustive answers to all the questions put forward by the selection process have yet to be found.

In this context, the purpose of our work is to provide a comparative assessment of the plus trees of Scots pine with respect to the formation and lignification of annual shoots of xylem.

2. Materials and Methods

The object of this research was to analyze the plus trees of Scots pine, which were allocated from natural plantings in the territory of the Nizhny Novgorod Region of the Russian Federation, according to the generally accepted methods of mass selection used in forest selection. Their vegetative offspring grew within the boundaries of the clone archive located in the same region, which is administratively part of a state budgetary institution of the Nizhny Novgorod Region, “Semenovskiy spetssemleskhoz” (specialized enterprise for the production of forest seeds and planting material). The location of the experimental site was 56°44'20'' N, 44°20'59'' E. This archive of clones was established in 1980 by landing 3-year saplings obtained by grafting cuttings of plus trees on well-developed 2-year-old rootstocks with a seed origin, which were cultivated in an open ground forest nursery of a specified forest-seed company. The characteristics of the soil in this land plot, with respect to its fertility, mechanical composition, and moisture conditions, were typical for the forest areas of the Central zone of the European part of Russia.

The studies were carried out by field stationary and laboratory methods, using such schemes as work organization and sampling construction, which allow for the implementation of the principles of the only logical distinction, reliability, suitability, and expediency of experience. The elimination of the differentiating influence of environmental factors was achieved by comparisons of the studied objects and samples (plants and their shoots), which were carried out only within the boundaries of one experimental site, on which uniform schemes, with respect to seating and plant feeding areas, were maintained. A single regime for maintenance and cultivation was supported, and the uniformity of the initial planting material (the same type of three-year-old grafted saplings) used for the creation of the clone archive was provided. The same silvicultural and agrotechnical measures were carried out. As a result, the experience in each of the work blocks was organized under the conditions of the aligned ecological background. The rows of clones were placed in their archives in accordance with the applicable regulations regarding the creation of objects of a constant forest-seed base and a single genetic-breeding complex. In particular, a randomized placement of rows on the plot was provided. This eliminated the targeted provision of any of the progenies of plus trees, including benefits in terms of growth and the formation of the plants, their shoots, and in the end, their physiological characteristics.

The elimination of time factor influence, causing chronographic (phenological and ontogenetic) variability of the analyzed characteristics of the studied plants, was achieved by the simultaneous harvesting of biological samples, simultaneous measurements, observation, and accounting of the analyzed parameters, and comparison of only same-age trees and their parts. In practice, this condition was achieved by simultaneous planting and by using of the same age planting material in the creation of such objects. Thus, the minimization of the influence of background factors on the phenotypic manifestation of physiological differences between plants allowed us to explain the presence of such differences as being due to the action of the hereditary specificity of the studied objects in relation to the analyzed features. The correctness of the procurement of the initial material was provided by a preliminary audit of the assortment structure of the surveyed archives of clones, during which the identification of each registration tree (ramet) was carried out, and it was established that they each belonged to a clone of a concrete plus tree (ortet).

The primary unit of a sample in the histochemical experience was represented by temporary preparations of cross-sectional cuts from the average part of the annual growth, which were analyzed using a microscope (Mikmed-2) after staining and fixation. The lignification of cellulose in the xylem cell walls was assessed using the traditionally applied qualitative reactions of phloroglucinol to lignin: a 10% solution of phloroglucinol in 95% ethanol, with the addition of a few drops of hydrochloric acid [70–75]. As a sighting control, we used slices that were not affected by the test reagents: the color of their xylem cells corresponded to the absence of lignification. These slices were compared to the slices treated with appropriate reagents, and were placed on the following scale: 0—no staining, no differences between the state of the painted slices and that of the control slices; 1—weakly expressed pale pink coloration; 2—well-fixed pale pink coloring; 3—medium-intensity pink coloring; 4—saturated pink coloration with an above average intensity, but not reaching the maximum; and 5—maximum intensity bright crimson coloration. For each zone, we counted the number of rows of cells in the radial direction, from the first row in the early xylem to the last visible row of the late xylem before cambium. The degree of xylem lignification was expressed as a percentage of the total number of rows of its cells. The ratio of early and late xylem zones was estimated by the balance of the number of cell rows in each of them at the end of the vegetation period, when all cells of late xylem underwent lignification. To construct an integral assessment of the state of xylem, we considered indicators that determine the degree of its development and the level of lignification in the observed sight sector, covering all accounting zones from the core to the cambium:

- The total number of rows of xylem cells formed at the time of accounting, which was determined by counting all rows of xylem cells, regardless of the fact and degree of their lignification (feature 1);
- The number of rows of completely lignified early xylem cells, which was determined by counting the rows of cells in the walls in which the process of lignification was fully completed, as confirmed by their uniform intense color, corresponding to an assessment of 5 points (feature 2);
- The number of rows of absolutely non-lignified xylem cells, determined by counting only those cells in the walls in which the signs of lignification were not fixed and there was no staining (i.e., assessment of 0 points) (feature 3);

- The number of rows of partially lignified xylem cells, determined by counting only those cells in which the process of wall lignification was fixed but not completely, as confirmed by their uniform color of different intensities from 1 to 4 points (feature 4);
- The depth of the lignification of xylem cells, taking into account the coefficient of their lignification grading, determined as the sum of the products of the number of cell rows in a separate accounting of each zone of xylem, on their lignification score (from 0 to 5 points), carried to the maximum assessment (5 points). This was expressed as the number of rows of xylem cells, corrected for the degree of lignification (feature 5);
- The absolute percentage of the lignification of xylem cells, which was determined as a percentage share of lignified xylem cells, in varying degrees (from 1 to 5 points), relative to their total number (feature 6);
- The relative percentage of xylem cell lignification, determined as a percentage share of fully and partially lignified xylem cells, the number of which was adjusted to the degree of their lignification (feature 7);
- The total sum of rows of the fully lignified (5 points) cells of early and late xylem (feature 8).

In order to obtain clear information concerning the importance of the actual analysis of the dynamics of plant physiological indicators and comparatively assess them, a static indicator fixed to the observation date was used. The sampling times were confined to the characteristic phenological plant states: the period of rest (the first period of accounting), the exit from the state of rest (the second period of accounting), the beginning of the formation of late xylem (the third period of accounting), the transition to the state of rest (the fourth period of accounting).

The actual material obtained in the course of the studies was processed statistically using ANOVA, according to the Snedekor algorithm [76–78]. Complex estimates of the relative genetic proximity or distance of the compared objects were obtained in the course of principal component analysis (PCA) and cluster analysis. These techniques are actively used in the study of tree species [79–81], including forestry research [82–84], the comparative assessment of plus trees [60,85], and the determination of genetic proximity [35,86,87]. An obligatory condition of the experiment was accepted (i.e., the presence of all plus trees of the same set of quantitative characteristics, the number of which determined the dimension of the Euclidean space). Clones that did not have at least one of the characteristics, or that did not receive reliable estimates during the preliminary statistical processing, were excluded from the scheme. The accepted order of the formation of numerical arrays made it possible to calculate the correlation and covariance estimates of each of the characteristics with all other object features, in a multidimensional comparison complex. Amendments to the unevenness of complexes were not entered, since the accounting of all parameters of the multidimensional objects in the surveyed site had the same number. In the calculations, we included the standardized values of the initial parameters, obtained by the z-transformation and based on the ratio of the deviation of the characteristic value from its average value to the corresponding standard deviation. As a result, a matrix of the normalized values of the features was obtained, which served as the basis for calculating the generalized statistical distances in Euclidean space. This approach yielded uniform and dimensionless characteristics, allowing the comparison of the object coordinates in the multidimensional feature space. The cluster analysis method was the ascending hierarchical procedure. The square of the Euclidean distance was used as a metric, and the agglomeration was considered as the average linkage (between groups).

3. Results

The assessment of the physiological status of the plus trees in clone archive No. 1, considering a wide complex of features characterizing the level of xylem development and the degree of cell lignification in the tissues of annual shoots, revealed a noticeable differentiation of the composition of the analyzed clones archive (Table 1).

Table 1. Average values of indicators of the physiological status of plus trees[1].

Number of plus trees	Features of xylem state (M±m)						
	Feature 1	**Feature 2**	**Feature 3**	**Feature 4**	**Feature 5**	**Feature 6**	**Feature 7**
Plus tree 14	24.33 ± 2.19	21.78 ± 2.27	1.67 ± 0.17	0.89 ± 0.31	0.60 ± 0.21	92.34 ± 1.34	91.04 ± 1.58
Plus tree 3	22.11 ± 1.18	20.33 ± 1.05	0.78 ± 0.15	1.00 ± 0.29	0.71 ± 0.20	96.44 ± 0.72	95.21 ± 0.58
Plus tree 19	29.56 ± 1.25	27.56 ± 1.21	1.00 ± 0.00	1.00 ± 0.29	0.73 ± 0.22	96.56 ± 0.18	95.67 ± 0.33
Plus tree 6	37.89 ± 1.80	35.56 ± 1.67	1.00 ± 0.00	1.33 ± 0.24	0.91 ± 0.18	97.31 ± 0.14	96.21 ± 0.21
Plus tree 22	21.78 ± 0.92	19.89 ± 0.89	1.00 ± 0.00	0.89 ± 0.26	0.60 ± 0.16	95.34 ± 0.20	94.02 ± 0.50
Plus tree 1	30.44 ± 2.68	28.56 ± 2.61	1.11 ± 0.11	0.78 ± 0.28	0.56 ± 0.20	96.13 ± 0.46	95.46 ± 0.45
Plus tree 5	19.78 ± 1.66	18.11 ± 1.60	1.11 ± 0.11	0.56 ± 0.24	0.40 ± 0.19	94.17 ± 0.56	93.38 ± 0.56
Plus tree 17	43.56 ± 4.61	41.11 ± 4.46	1.33 ± 0.17	1.11 ± 0.26	0.78 ± 0.20	96.85 ± 0.33	96.04 ± 0.36
Plus tree 18	31.67 ± 1.00	29.67 ± 0.94	1.00 ± 0.17	1.00 ± 0.29	0.78 ± 0.26	96.83 ± 0.51	96.10 ± 0.48
Plus tree 40	23.33 ± 0.80	21.44 ± 0.91	1.00 ± 0.00	0.89 ± 0.31	0.67 ± 0.25	95.68 ± 0.14	94.71 ± 0.35
Plus tree 41	34.56 ± 1.16	32.33 ± 1.25	1.00 ± 0.00	1.22 ± 0.28	0.93 ± 0.23	97.08 ± 0.09	96.22 ± 0.24
Plus tree 45	22.78 ± 1.02	21.00 ± 1.09	0.89 ± 0.11	0.89 ± 0.26	0.60 ± 0.19	95.93 ± 0.52	94.68 ± 0.64
Plus tree 47	24.78 ± 2.41	22.56 ± 2.33	1.00 ± 0.00	1.22 ± 0.22	0.87 ± 0.17	95.70 ± 0.36	94.25 ± 0.50
Plus tree 29	31.78 ± 2.34	29.44 ± 2.29	1.33 ± 0.17	1.00 ± 0.24	0.73 ± 0.17	95.72 ± 0.49	94.89 ± 0.64
Plus tree 49	23.78 ± 3.14	21.67 ± 3.12	1.00 ± 0.00	1.11 ± 0.26	0.87 ± 0.24	95.23 ± 0.57	93.98 ± 0.86
Plus tree 39	21.00 ± 0.82	19.00 ± 1.11	1.00 ± 0.00	1.00 ± 0.37	0.78 ± 0.31	95.18 ± 0.18	94.04 ± 0.65
Plus tree 21	32.56 ± 2.10	30.44 ± 1.82	1.11 ± 0.11	1.00 ± 0.33	0.73 ± 0.26	96.53 ± 0.30	95.79 ± 0.24
Plus tree 20	21.44 ± 1.33	19.33 ± 1.25	1.00 ± 0.17	1.11 ± 0.31	0.80 ± 0.22	95.07 ± 1.03	93.72 ± 0.98
Plus tree 30	31.00 ± 1.40	28.78 ± 1.30	1.00 ± 0.17	1.22 ± 0.22	0.87 ± 0.15	96.77 ± 0.48	95.64 ± 0.36
Plus tree 42	34.22 ± 3.02	31.89 ± 2.77	1.11 ± 0.11	1.22 ± 0.55	0.91 ± 0.43	96.60 ± 0.33	95.70 ± 0.61
Plus tree 44	27.56 ± 1.26	25.56 ± 1.31	0.89 ± 0.11	1.11 ± 0.26	0.69 ± 0.15	96.74 ± 0.44	95.11 ± 0.86
Total	28.09 ± 0.62	26.00 ± 0.60	1.06 ± 0.03	1.03 ± 0.06	0.74 ± 0.05	95.91 ± 0.14	94.85 ± 0.16

[1] The most informative features. Abbreviations of the features are used as specified in the Methods section. M: the average value of the feature; ± m: error of the representativeness of the sample mean.

Since the fixed phenotypic differences between the plus trees appeared while the effects of environmental conditions were controlled for, there are grounds to consider the cause of their occurrence as predominantly genotypic. Statistical confirmation of this was obtained in the course of the analysis of variance, in which the actual value of Fisher's F-criteria for most features exceeded the minimum allowable limit, with 5% and 1% significance levels. Significant differences failed to be confirmed in only two cases (according to features 4 and 5). We confirmed the effect of the differences between the actual plus trees (ortets), which in such cases is considered as the coefficient of heritability in the broad-sense, on signs, concerning the essentiality of the differences. This effect reached values between 16.85% ± 9.90% for feature 1 to 49.59% ± 6.00% for feature 2, with the general background of phenotypic differences.

The statistical reliability of the obtained results and the presented evidence of the hereditary conditionality of the revealed phenotypic variability of plus trees with respect to the physiological state of xylem of their annual shoots created sufficient grounds for conducting multivariate analyses. In the factor analysis (PCA), these characteristics were grouped according to the principal components. The results of this transformation are reflected in Figure 1 and in Table 2.

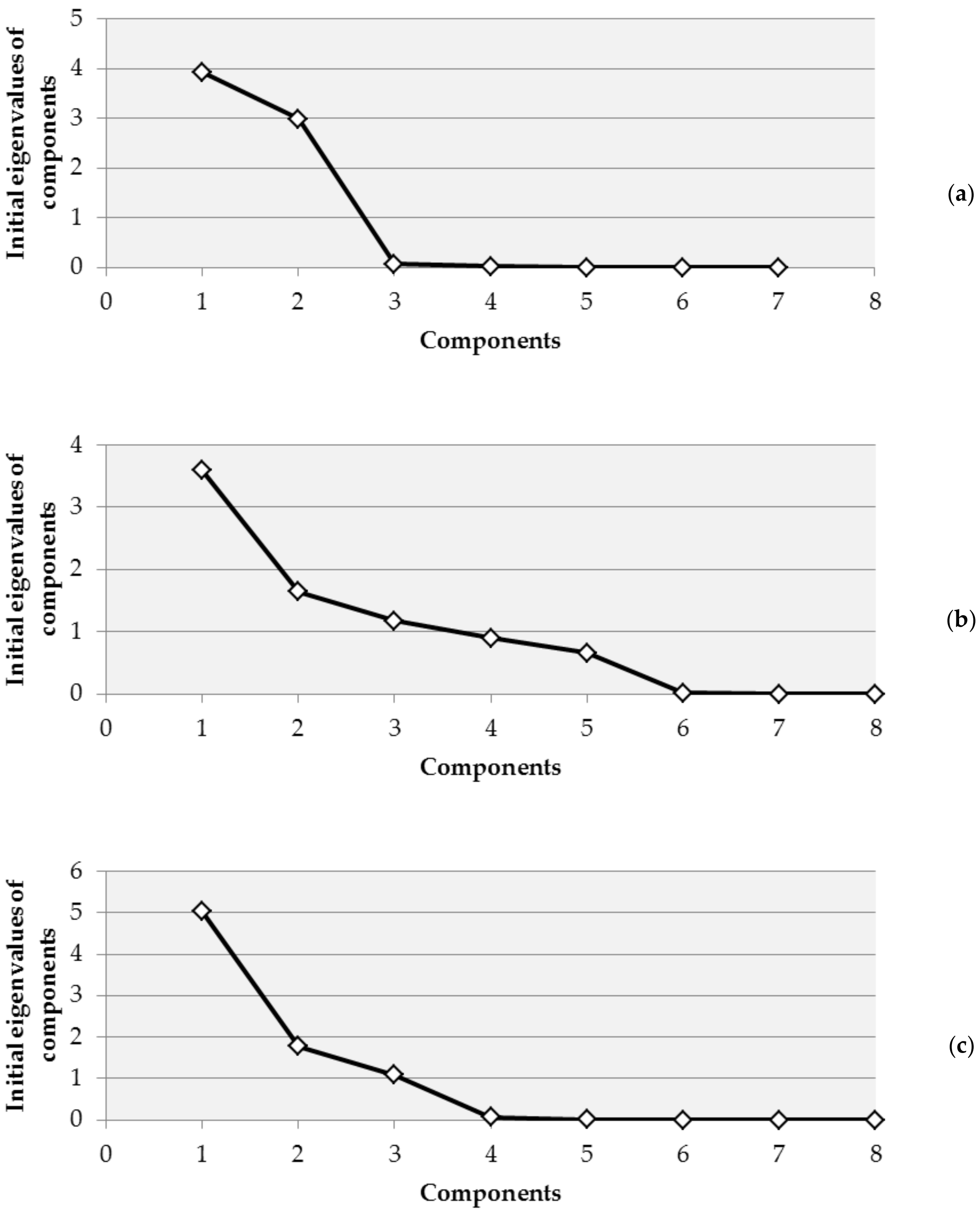

Figure 1. Initial eigenvalues of the components with respect to the time of accounting of the plus tree xylem states: (**a**) January; (**b**) April; (**c**) July.

Figure 1 displays the extraction of the principal components, and indicates quite a satisfactory outcome of this variant of factor transformation. The distribution of analyzed variables (values of analyzed traits) is clearly visible, systemic, and logical (see Table 2). Note that features 4 and 5, as well as features 6 and 7, were always part of the same components. In the formation of the principal components, feature 1 always participated in combination with feature 8, and feature 3 with features 6 and 7. At the same time, some specificity of the structure of the principal components was revealed on each of the observation dates. Note that information on feature 2 in the first term of registration is not presented due to the absence of the number of rows of fully lignified cells of early xylem formed in the current year at the time of registration of the actual indicator. The general trend in the procedure of extracting the principal components was, for all accounting periods, a convincing cut-off of insignificant values (values close to 0; see Figure 1).

Table 2. Structure of the principal components of the xylem state of plus trees in terms of the accounting period[1].

Signs and indicators	Principal components of the accounting period							
	the first period		the second period			the third period		
	first	second	first	second	third	first	second	third
Feature 1	0.944	-	-	-	0.793	0.962	-	-
Feature 2	-	-	-0.794	-	-	0.966	-	-
Feature 3	-	-0.972	-	0.847	-	-	-	-0.931
Feature 4	0.982	-	0.864	-	-	-	0.936	-
Feature 5	0.989	-	0.962	-	-	-	0.939	-
Feature 6	-	0.986	-	-0.838	-	-	-	0.763
Feature 7	-	0.980	-	0.684	-	-	-	0.713
Feature 8	0.980	-	-	-	0.721	0.966	-	-
Initial eigenvalues of principal components								
Value	3.9214	2.9845	3.5994	1.6475	1.1740	5.0405	1.7811	1.0901
The share of the variance of principal components, %								
Dispersion	56.020	42.635	44.992	20.594	14.675	63.007	22.264	13.627
Cumulative variance of principal components, %								
Dispersion	98.655		80.261			98.897		

[1]The rotated components of factor analysis are presented herein.

The sufficient stability and the uniformity of the principal components formation, in terms of the accounting of the seasonal state of xylem during the year, allowed factor analysis (PCA) to be carried out on a generalized data set (Figure 2, Table 3).

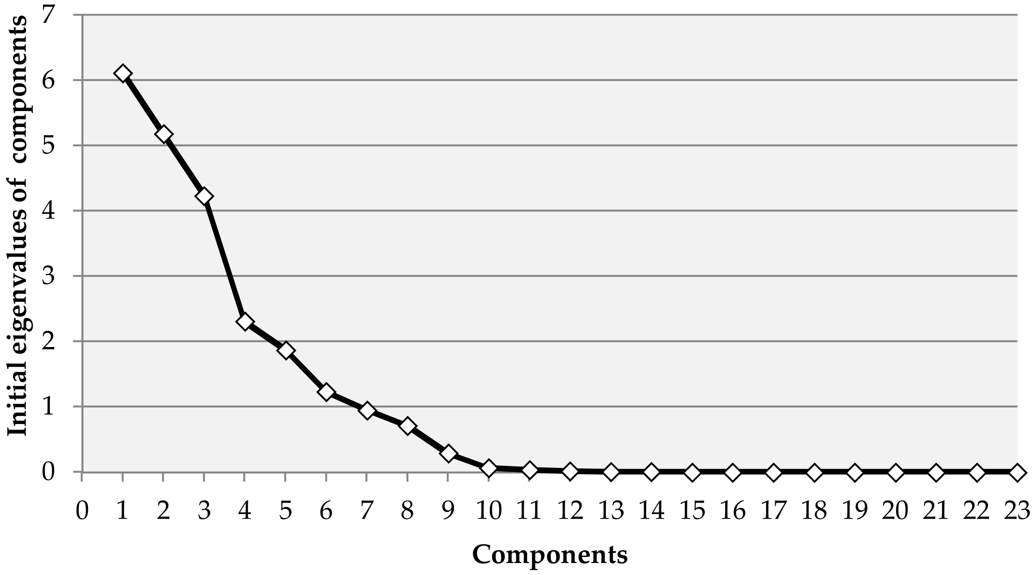

Figure 2. Initial eigenvalues of the components for all periods of accounting of the seasonal state of xylem in the shoots of plus trees.

Figure 2 illustrates the allocation of six principal components from the full list of traits. Their structure is revealed by the materials shown in Table 3, in which the distribution of the variables under consideration are determined, predominantly seasonally, by the independent factors (principal components).

Table 3. Structure of the principal components for all accounting periods[1].

Features of the plus trees	Principal components of factor analysis on the seasonal state of xylem					
	first	second	third	fourth	fifth	sixth
Feature 1	-	0.9317	-	-	-	-
Feature 2	-	-	-	-0.9550	-	-
Feature 3	-	0.9736	-	-	-	-
Feature 4	-	0.9786	-	-	-	-
Feature 5	-	-	-	0.9641	-	-
Feature 6	-	-	-	0.9473	-	-
Feature 7	-	0.9738	-	-	-	-
Feature 8	-	-	-	-	0.6315	-
Feature 9	-	-	-0.7404	-	-	-
Feature 10	-	-	-0.7769	-	-	-
Feature 11	-	-	0.8779	-	-	-
Feature 12	-	-	0.7534	-	-	-
Feature 13	-	-	0.7449	-	-	-
Feature 14	-	-	-0.4339	-	-	-
Feature 15	0.9785	-	-	-	-	-
Feature 16	0.9733	-	-	-	-	-
Feature 17	0.9778	-	-	-	-	-
Feature 18	-	-	-	-	0.8805	-
Feature 19	-	-	-	-	-	0.7950
Feature 20	-	-	-	-	-	0.8085
Feature 21	0.7383	-	-	-	-	-
Feature 22	0.7954	-	-	-	-	-
Feature 23	0.9785	-	-	-	-	-
Initial eigenvalues of principal components						
Value	6.1104	5.1788	4.2336	2.3068	1.8718	1.2290
The share of the variance of principal components, %						
Dispersion	26.567	22.516	18.407	10.029	8.138	5.343
Cumulative variance of principal components, %						
Dispersion	91.001					

[1] The rotated components of factor analysis are presented herein.

The material obtained at this stage of the work made it possible to carry out cluster analysis, with the use of both normalized values of the initial features (Table 4, Figure 3) and the principal components derived on their basis (Table 5, Figure 4).

Table 4. The scheme of the clustering of plus trees with respect to the initial indicators of the physiological state of xylem.

Steps of agglomeration			Distance of cluster combine		Stages of appearance first cluster		The next step
Step	Cluster's combine		coefficients	25-bit scale			
	first	second			cluster 1	cluster 2	
1	10	15	8.387	3.21	0	0	5
2	4	19	9.090	3.48	0	0	6
3	3	14	10.908	4.17	0	0	6
4	9	21	13.657	5.23	0	0	10
5	5	10	14.564	5.57	0	1	9
6	3	4	14.909	5.70	3	2	7
7	3	11	16.473	6.30	6	0	12
8	13	16	17.654	6.76	0	0	19
9	5	12	18.472	7.07	5	0	13
10	6	9	19.863	7.60	0	4	14
11	17	20	23.757	9.09	0	0	17
12	3	8	25.041	9.58	7	0	14
13	5	7	26.048	9.97	9	0	16
14	3	6	29.774	11.39	12	10	16
15	2	18	36.813	14.09	0	0	17
16	3	5	38.921	14.89	14	13	18
17	2	17	44.489	17.02	15	11	18
18	2	3	54.443	20.83	17	16	19
19	2	13	62.215	23.81	18	8	20
20	1	2	65.333	25.00	0	19	0

The implementation of the clustering procedure was completed by the construction of a hierarchical system in which the plus trees were grouped according to the criterion of the greatest similarity, by the analyzed complex of the signs of the physiological state of xylem (see Table 4, Figure 3).

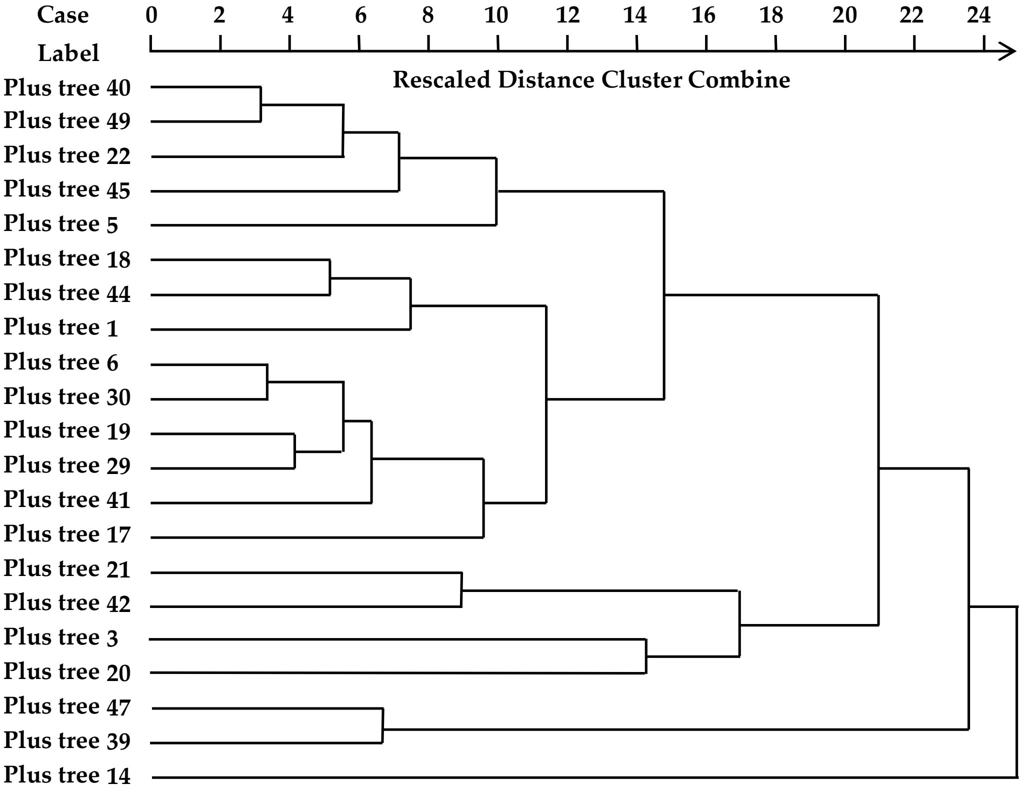

Figure 3. Dendrogram of the similarity between plus trees, built according to the normalized values of the source characteristics of the physiological state of the xylem.

In its structure, there is a well-formed macro-cluster containing plus trees with numbers 40, 49, 22, 45, 5, 18, 44, 1, 6, 30, 19, 29, 41, and 17. It has noticeable differences from the others in terms of the connection distances. Its own structure is represented by three micro-clusters of roughly the same density of addition, but with different numbers.

The dendrogram built on the basis of the principal components of the considered set of features (see Table 5, Figure 4) also had a pronounced hierarchical constitution.

Table 5. The scheme of the clustering of plus trees with respect to the principal components of the physiological state of xylem.

Steps of agglomeration			Distance of cluster combine		Stages of appearance first cluster		The next step
Step	Cluster's combine		coefficients	25-bit scale	cluster 1	cluster 2	
	first	second					
1	5	15	1.037	1.23	0	0	3
2	4	19	1.287	1.53	0	0	7
3	3	5	1.844	2.19	0	1	5
4	9	21	2.134	2.54	0	0	15
5	3	10	2.394	2.85	3	0	9
6	13	16	3.289	3.91	0	0	18
7	4	11	3.724	4.43	2	0	8
8	4	14	4.632	5.51	7	0	13
9	3	12	4.668	5.55	5	0	11
10	6	17	6.025	7.16	0	0	14

11	3	7	6.419	7.63	9	0	13
12	18	20	7.253	8.63	0	0	15
13	3	4	7.789	9.26	11	8	16
14	6	8	8.559	10.18	10	0	17
15	9	18	9.208	10.95	4	12	16
16	3	9	10.946	13.02	13	15	17
17	3	6	12.410	14.76	16	14	18
18	3	13	14.435	17.17	17	6	19
19	2	3	16.041	19.08	0	18	20
20	1	2	21.022	25.00	0	19	0

At the same time, while maintaining a certain specificity in each of the analysis options, the structures of these dendrograms had a fundamental similarity. Note that the plus trees marked by the indices 40, 49, 22, 45, and 5, located on the first dendrogram (built on the basis of the normalized values of 23 initial features) in close proximity to each other, preserve the similarity of positions on the second dendrogram (built on the basis of 6 principal components). Separate pairs of objects, such as plus tree 18 and plus tree 44, plus tree 6 and plus tree 30, and plus tree 47 and plus tree 39, were found on both the first and second dendrograms. In this case, in each of the variants of the analysis, plus tree 47 and plus tree 39, were among those that had the largest values with respect to the distance of the hierarchical cluster combination. The most specific object, plus tree 14, remained so on each of the received dendrograms.

The obtained material allowed the fundamental similarity of the graphical representations of the structure of the relations between plus trees in the multidimensional space of traits to be recognized. This indicates that the observed ratio between the plus trees on the characteristics of the xylem state has a stable character. The presence of plus trees, which have characteristics that largely distinguish them from others, has predetermined that these plus trees are potentially unrelated to the main composition of the specified archive of clones.

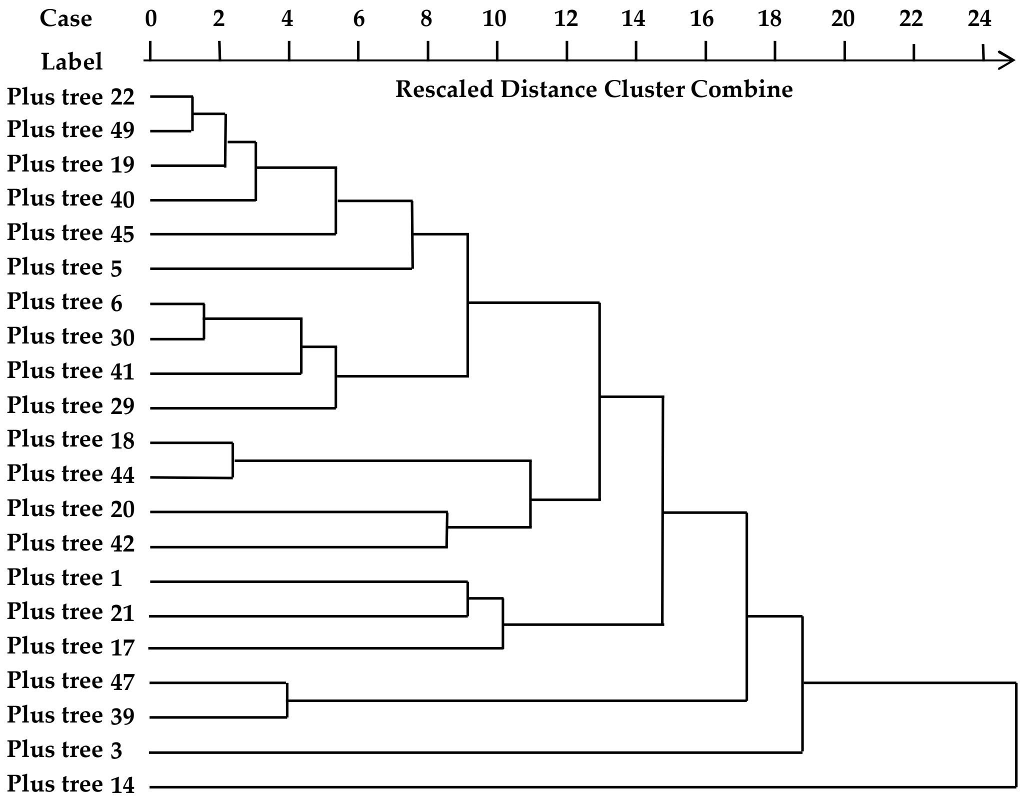

Figure 4. Dendrogram of the similarity between plus trees, built on the basis of the principal components of physiological assessments of the seasonal state of xylem.

4. Discussion

The power of the xylem zone at each stage of the vegetation period was determined by the number of cell layers within it. On average, the vegetative offspring of the studied plus trees of Scots pine formed 28.09 layers of xylem cells during the growing season in seed orchard conditions. The specific phases of seasonal development of the studied plants corresponded to quite a definite number of xylem cell layers. In particular, the completion of the formation of early xylem occurred when 26.00 of its cellular layers were formed. The appreciable phenotypic heterogeneity of the plus trees of Scots pine is revealed, with respect to their ability to form a number of xylem cell layers in their shoots during the vegetation period. According to this indicator, three groups of objects in the assortment composition of the analyzed archive of clones were allocated. The most cell layers were in the xylem-formed in clones of plus tree 17 (43.56 layers) and plus tree 6 (37.89 layers), and the fewest were found in the clones of plus tree 5 (19.78 layers), plus tree 39 (21.00 layers), and plus tree 20 (21.44 layers). The absolute maximum of this characteristic (63 layers) was 5.73 times higher than the absolute minimum (11 layers). The most numerous group was that with average, or approximately average, values (28.09 layers). Trees capable of forming more cellular layers during the vegetation period are of greater interest in the selection of increased wood growth and overall high biomass increment. This is a priority in forestry plantation. The indicator used herein can be used in the selection assessment of plus trees.

Lignification, accompanying the formation of new layers of xylem cells in the annual shoots of plus trees, determines how ready plants are for the period of adverse winter conditions. The rate of its flow and the limits of its values achieved in this case are associated with the phases of the formation of escape zones. The compared objects had different completion times for the lignification of early xylem and its level, while the simultaneous assessment during this period indicated that there were also differences in the timing and extent of the final completion of lignification. The highest lignification rates of xylem cells were found in the plus trees with indices 6 (97.31%) and 41 (97.08%), and the smallest rates were found in the plus trees with index 14 (92.34%). The former had the ability to complete all of the processes relating to the formation of wood shoots in a relatively short time, with a minimum duration during the vegetation period. The latter required a longer duration of shoot growth for its normal development. The former are better adapted to the conditions of a relatively short summer, and the latter to a longer one. Those plus trees that fully completed the xylem lignification processes by the end of vegetation are more adapted to adverse winter conditions. On this basis, they can be considered as the most promising for the selection.

The marked heterogeneity of the physiological state of the vegetative offspring of plus trees was largely (up to 53% and higher) due to genotype, as confirmed by the ANOVA results. The estimates obtained in the course of its implementation corresponded to the levelling of the growth conditions and the corresponding minimization of the influence of external factors on the phenotypic differentiation of the analyzed plants, according to the considered indices. However, the development of xylem (i.e., the rate and level of xylem cell lignification in the shoots of plus trees) is dependent on environmental factors to some extent. Their influence ranged from 22.16% (number of rows of fully lignified cells of early xylem) to 88.98% (lignification depth of xylem cells), and did not prevail in all cases. Their influence can neutralize the difference in the indicators with a genotypic nature and predict the possibility of sufficiently large changes in the phenotypic manifestations of the trait. The physiological variance component, associated with the differences between the clones (ramets), was estimated to be between 6.90% and 23.98%. Its occurrence was mainly due to the technological diversity of grafting. The indices of development and lignification of xylem can serve as an indicator for the early diagnosis of selected trees according to the rate of tree weight gain and selection estimation of clones.

The degree of the non-coincidence of the physiological characteristics of the plus trees was not the same, which made it possible to identify the groups of objects that were relatively similar to each other in their composition throughout the set of analyzed indicators (i.e., the clusters). At the same time, there were well-marked differences between such groups. The factor analysis allowed the number of indicators to be reduced, while maintaining the information content of the original complex in principle. The results of the dendrogram's construction on the basis of the principal components formed were quite similar to the results of their construction according to the primary data. The cluster analysis allowed a natural grouping of the assortment of plus trees to be performed according to the similarity criteria of the seasonal state estimates of their xylem. As a result, the groups of plus trees with relatively similar characteristics were established with respect to the whole list of estimations of the development and lignification of xylem cells. The objects that were most distant from the others with respect to their studied population were also revealed. This information provides a reasonable approach to the formation of such an assortment of Scots pine seed orchards, which minimizes the risk of inbreeding depression.

5. Conclusions

The plus trees of Scots pine are heterogeneous with respect to the physiological state of their xylem. To a large extent, their specific abilities to form xylem zones with different thicknesses during one vegetation period is determined genotypically. The rate of annual shoot xylem cell lignification is also influenced by heredity. Carriers of these signs are most preferred in the composition of seed orchards. In composing clone archives, the ability to select a group of plus trees (and particular plus trees) that are the most different from the others with respect to their complexes of physiological characteristics is essential. The hereditary conditionality of the latter allows us to accept a position, according to which such plus trees have a minimum chance of being closely related in their origin. The use of this approach in the creation of seed orchards can reduce the risk of inbreeding depression in seed offspring and allows its negative effects to be weakened.

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