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INDIVIDUAL DIFFERENTIATION OF *ABIES ALBA* MILL. POPULATION FROM THE TISOVIK RESERVE. VARIABILITY EXPRESSED IN MORPHOLOGY AND ANATOMY OF NEEDLES

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One-year-old needles from 20 silver fir trees in the Tisovik Reserve (Belarus) were characterized in respect to 12 morphological and anatomical traits, and the data were analyzed statistically to determine variability between trees. Individuals within the population generally were homogenous for those traits. Needle length was the most variable trait within the studied population. The width/height ratio of the hypodermic cell exerted the weakest differentiating effect between trees. The results indicated that the Tisovik Reserve population is weakly differentiated.

Key words: European silver fir, variability, needle, multivariate statistical analysis.

INTRODUCTION

European silver fir (*Abies alba* Mill.) is one of the most important trees in Central European forests. It is a mountain tree but sometimes it occupies lowlands, especially in the northern parts of its distribution. In Europe, silver fir occurs in smaller and bigger groups following the distribution of mountain ranges. The geographical distribution of silver fir is limited mainly to European mountains: the Alps, Carpathians, Apennines and Pyrenees. The distribution limit of *A. alba* reaches west to the Pyrenees, east to Stara Planina in Bulgaria and the Southern Carpathians in Romania, and south to the Aspromonte Mts in the south of the Apennine Peninsula. In the north, silver fir occurs in small isolated populations in the Middle European Depression, in the Tisovik Reserve in the Białowieża Primeval Forest (Belarus).

The isolated population in the Białowieża Primeval Forest is the subject of this research. The population was discovered by Górski in 1823 (Górski, 1829). He found it in Cisovka Hag, a small forest island surrounded by widespread bogs called Dikij Nikor. The uniqueness of the Cisovka silver fir population is its locality. This population is isolated and farthest from the continuous range of European silver fir, 120 km northeast of it (Szafer, 1920).

According to Górski (1829) and Szafer (1920) it is a native and relict population of European silver fir, but some authors questioned the natural origin of silver fir from Cisovka (Trauwetter, 1850, cited in Szafer, 1920; Graebner, 1918; Karpiński, 1933). On the basis of an analysis of the genetic structure, Mejnartowicz (1996) supplied documentary evidence that this population's origin is native and natural. Korczyk et al. (1997) argued the same on the basis of an analysis of demographic structure. They also confirmed that silver fir has been growing uninterruptedly for 300 years at this stand. This paper is another contribution to the discussion of the

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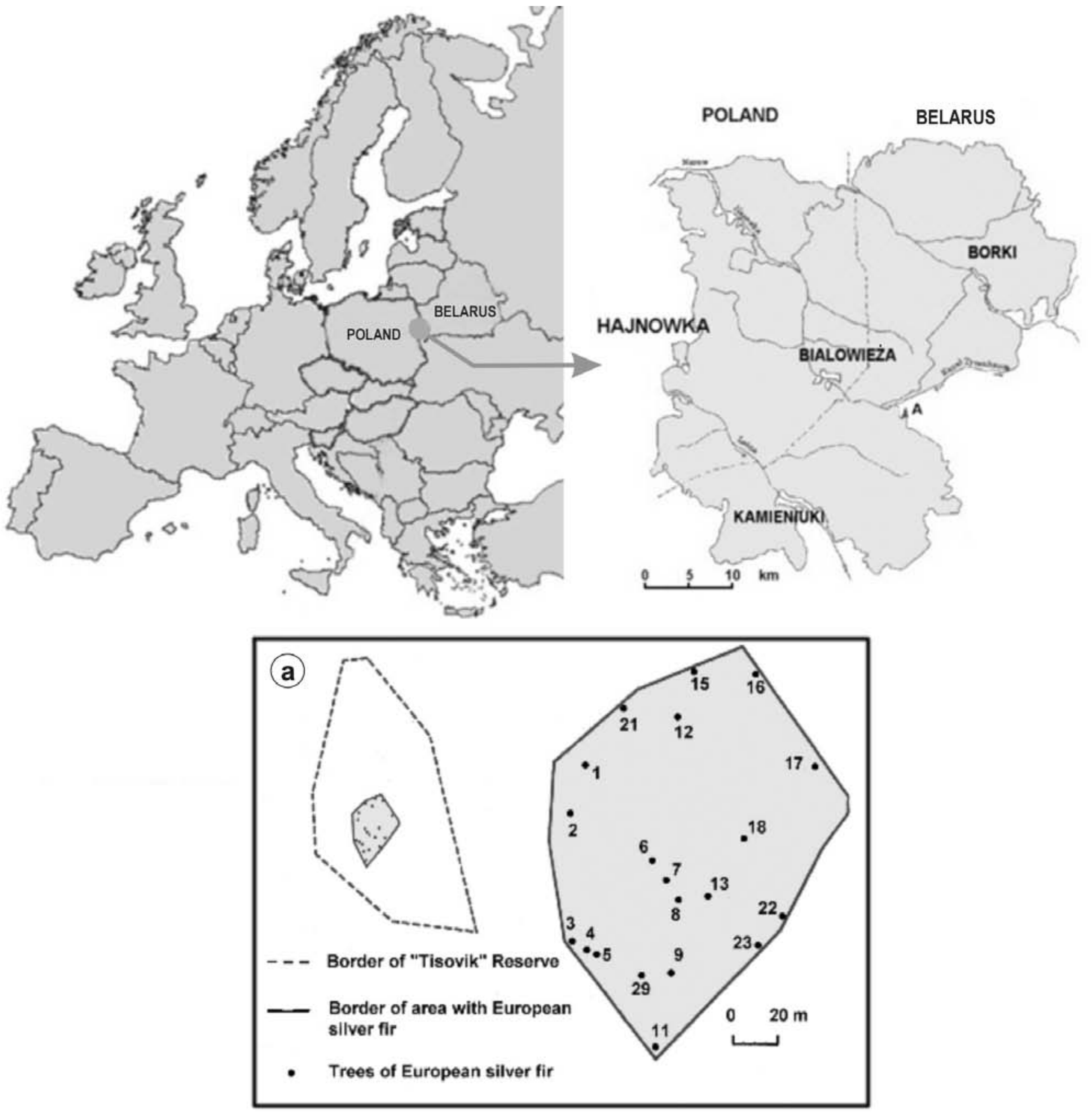

Fig. 1. Location of the Białowieża Primeval Forest and the Tisovik Reserve, (**a**) Tisovik Reserve, and silver fir area (according to Korczyk et al., 1997; Goncharenko and Savitsky, 2000).

natural or artificial origin of this species in the Białowieża Primeval Forest.

Up to 1939, Cisovka Hag belonged to Poland. On the basis of Szafer's initiative of 1920, the Polish Committee for Nature Protection proposed the creation of the Cisovka Reserve for preservation of the silver fir. This reserve was founded in 1921 and existed until World War II. After the war, the larger part of Białowieża Primeval Forest as well as the Cisovka Reserve became part of Belarus. Cisovka was renamed Tisovik. Drainage of the Dikij Nikor bogs and pasturing of cattle and sheep began at that time. These activities caused severe losses in the silver fir stand. In 20 years the number of silver fir sharply decreased. In 1958, Zefirov (1958) found only 36 firs, whereas in 1887 Błoński et al. (1888) had found several hundred firs. Recently the number of silver fir trees in the population has reached a critical level. Twenty silver firs are growing in the reserve at present (Korczyk et al. 1997). The Cisovka/Tisovik Reserve is a declining population which could go through a bottleneck and increase in numbers or else completely disappear as a result of aggravated environmental conditions (Shutko and Martinovič, 1967; Budničenko et al., 1987; Mejnartowicz, 1996). In 1991 the Tisovik Reserve was incorporated into the Belarusan National Park, and the silver fir of the Białowieża Primeval Forest was registered as a species threatened with extinction in the Belarusan red book.

Because there are so few silver fir trees in the Tisovik Reserve, the need for research on preservation of its gene resources is urgent. With this imperative in mind, our investigation is aimed at describing the intrapopulational structure of silver fir from the Tisovik Reserve. The genetic variability of silver fir from the Tisovik Reserve was described earlier by means of isoenzymatic methods (Mejnartowicz, 1996; Goncharenko and Savitsky, 2000), but the variability of morphological and anatomical traits of the needle has not been determined before.

Generally speaking, little is known about the morphological and anatomical variability of *Abies alba*, unlike other conifers, especially Scots pine (*Pinus sylvestris*). The latter has been described in many papers (Bobowicz, 1990; Bobowicz and Korczyk, 1990, 1994; Bobowicz, 1993; Bobowicz et al., 1995, 2001a,b; Pawlaczyk et al., 1999). A paper on variability expressed in needle traits of *Abies alba* (Pawlaczyk et al., 2002) is one of the very few papers about the morphological and anatomical variability of this species. That study was limited to three typical populations growing at higher elevations. It does not portray the differentiation of untypical, isolated populations like the one in the Tisovik Reserve. The aim of the present investigation was to describe the morphological and anatomical variability of silver fir from the Tisovik Reserve.

MATERIALS AND METHODS

MATERIAL

The Tisovik Reserve is situated about 0.6 km from the eastern edge of the Białowieża Primeval Forest (Fig. 1), on wide expanses of drained bogs at Dikij Nikor (52˚40' N, 23˚50' E). This reserve occupies territory at 150 m a.s.l. and covering about 14 ha. The highest point of the reserve is about 80 cm above the level of an adjacent pasture. This area has shrunk considerably in the last 100 years; in 1888 it covered about 22 ha (Korczyk et al., 1997). In this reserve there are only 20 trees, whose distribution is shown in Figure 1.

The land is covered by turf-podzolic soil. In the 1950s a drainage scheme was carried out in the Tisovik area, which resulted in soil podzolization (Utenkova and Romanovski, 1972).

A phytosociological analysis of this reserve demonstrated that the plant communities in the whole area of the reserve are little differentiated and represent a subassembly of typical dry *Tilio-Carpinetum typicum* forest (Korczyk et al., 1997). The tree stand consists of hornbeam, oak and spruce. Silver fir, small-leaved lime, maple, mountain elm and ash are additional species in this tree stand.

The silver firs in this reserve are from 28 to 42 m high and show conspicuous set-back in height. The thickness of these firs is from 43.5 to 87.0 cm dbh. The dynamics of growth are clearly differentiated between particular trees. Height accretion has varied from 1.5 cm to 6.5 cm during the last seven years (Korczyk et al., 1997).

The age of 11 silver fir trees was from 106 to 154 years. The oldest silver fir was 154 years old and measured 82 cm dbh and 32 m in height (Korczyk, 1998). In view of the need for conservation, not all the trees have been drilled (Korczyk et al., 1997).

The condition of these trees was estimated on the basis of defoliation. Needle loss ranges from 15% to 35% in particular trees, and the average is 29.2%. Research from 1992–1998 indicates that this population is in its terminal phase of degradation.

BIOMETRIC METHODS

One-year-old needles from 20 trees of *Abies alba* Mill. were used to make slides (cross section of needles) and then analyzed for 12 morphological and anatomical traits: (1) needle length, (2) diameter of resin canals, (3) needle width measured in cross section, (4) needle height measured in cross section, (5) hypodermic cell height, (6) hypodermic cell width, (7) number of endodermic cells around vascular bundle, (8) distance from vascular bundle to needle edge, (9) distance from resin canals to needle edge, (10) distance from resin canals to vascular bundle, (11) needle height-to-width ratio (traits 4/3), (12) hypodermic cell width-to-height ratio (traits 6/5) (Fig. 2).

The first trait was measured with graph paper, and the others with the use of a light microscope. Traits 3 and 4 were measured under 4× magnification, and traits 2, 5, 6, 8, 9 and 10 were measured under 40× magnification.

STATISTICS

The data for the 12 morphological and anatomical traits of needles were analyzed with Statistica ver. 5.1 (StatSoft Inc.) The analyses were based on trait characteristics (Williams, 1995; Ferguson and Takane, 2003), correlation coefficients between traits (Łomnicki, 2002), Student's t-test (Fisher, 1925; Triola, 1998), discriminant analysis (Krzyśko, 1990), Mahalanobis distances between trees, Hotelling T^2 statistics (Hotelling, 1957; Krzyśko 1990) and agglomerant grouping on the basis of Euclidean distances (Sokal and Rohlf, 1997).

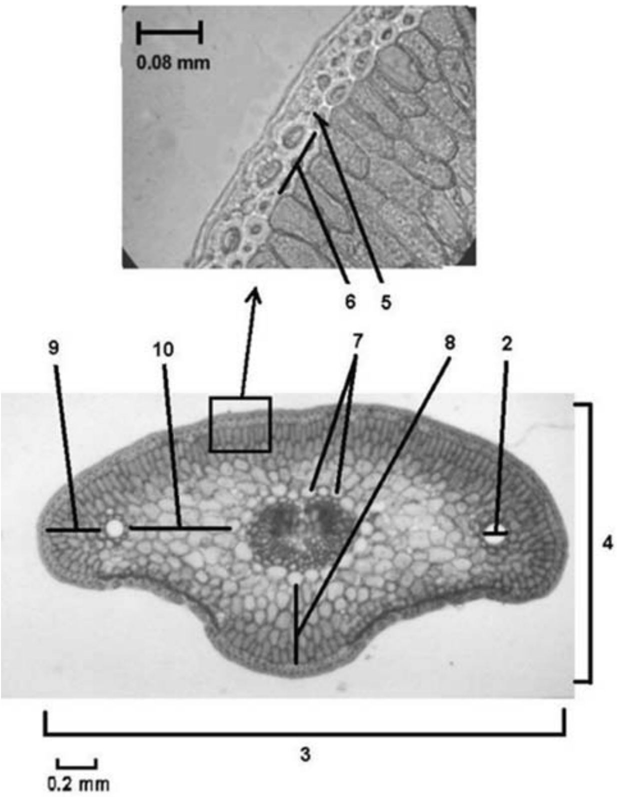

Fig. 2. Cross section of silver fir needle. Numbers 2 to 10 denote the measured anatomical traits.

TABLE 1. Trait characteristics: means ($\overline{X}$), standard deviations (s), variability coefficients (V%), minima and maxima of 12 morphological and anatomical needle traits (1-12) of *Abies alba* Mill. from the Tisovik Reserve

Trait	$\overline{X}$	s	V%	min	max
1	16.795	2.521	15.01	12.00	23.00
2	0.121	0.02	18.82	0.07	0.20
3	2.122	0.15	7.28	1.74	2.48
4	1.084	0.10	9.37	0.79	1.33
5	2.150	0.332	15.46	1.30	3.12
6	2.062	0.350	16.99	1.30	3.38
7	25.52	2.603	10.20	19.0	33.0
8	0.309	0.04	12.60	0.21	0.41
9	0.223	0.04	17.01	0.12	0.37
10	0.335	0.05	14.36	0.20	0.47
11	0.512	0.044	8.59	0.35	0.63
12	0.978	0.207	21.22	0.54	1.83

TABLE 2. Determination coefficients between the 12 morphological and anatomical needle traits of silver fir from the Tisovik Reserve and the first two discriminant variables, U_1 and U_2

Trait	U_1 50.64%	U_2 20.85%
1	40.3606	1.1505
2	0.0183	0.8569
3	2.2967	12.7049
4	3.3883	9.0211
5	0.0556	0.3977
6	0.1864	0.0082
7	1.1206	1.5085
8	1.8298	3.6157
9	1.1916	8.7500
10	0.6166	0.1438
11	9.3691	0.1330
12	0.0452	0.1236

RESULTS

Mean values, standard deviations, variability coefficients, minima and maxima for the 12 needle traits are presented in Table 1. None of the traits exceed 21.22% variability, and none are below 7.28%. The highest variability among simple traits, 18.82%, is for resin canal diameter (2), and the lowest variability among simple traits, 7.28%, is for needle width (3) (Fig. 2)

The correlation coefficients among the 10 simple morphological and anatomical traits were calculated. Of all 45 correlation coefficients, only 3 (6.67%) are highly significant ($\alpha = 0.01$), and 12 (26.67%) are significant ($\alpha = 0.05$).

For the Tisovik population the strongest correlation is between needle height (4) and distance from vascular bundle to needle edge (8), with an absolute value of 0.91 for this coefficient. The weakest correlation is between number of endodermic cells around vascular bundle (7) and distance from vascular bundle to needle edge (8) (Fig. 2).

To check the significance of differences in needle traits between individual trees, the Student t distribution was calculated. On the basis of the distribution, Student t-tests were performed separately for each of the studied needle traits (unpublished data). These tests indicated that the traits most differentiating the trees within the population are needle length (1), needle width (3), needle height (4) and needle height/width ratio (11). The least differentiated traits were the hypodermic cell width/height ratio (12), hypodermic cell width (6) and hypodermic cell height (5) (Fig. 2).

Variability within the studied silver fir population is presented in a discriminant analysis graph, a minimum spanning tree (drawn on the basis of the shortest Mahalanobis distances) and a dendrogram (Figs. 3–5).

Multitrait variability within the studied population of silver fir was detected by discriminant analysis. The results on the plane of the first two discriminant variables are presented in Figure 3, which shows that the trees within the analyzed population do not form groups.

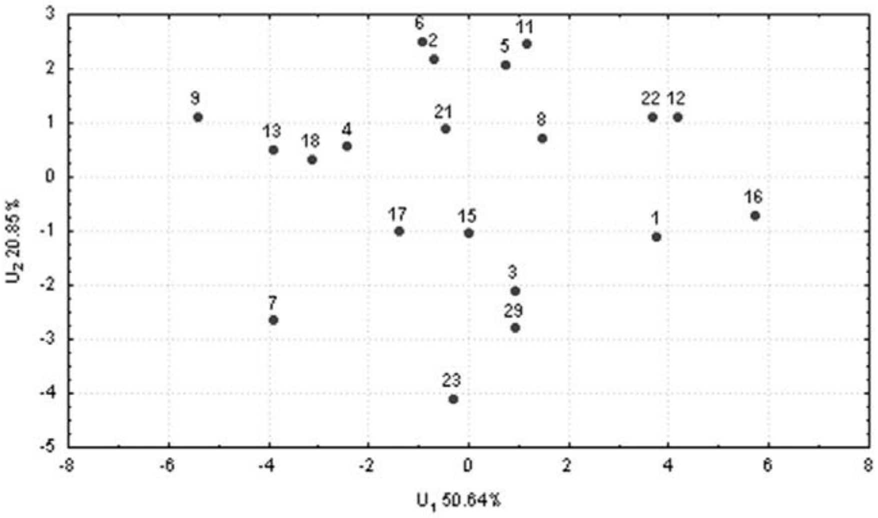

Fig. 3. Results of discriminant analysis of 20 silver fir trees from the Tisovik Reserve population, on the plane of the first two discriminant variables (U_1 and U_2), accounting for 71.49% of the variability of the 12 studied morphological and anatomical traits of needles.

On the basis of determination coefficients (Tab. 2) between the 12 needle traits and between the first two discriminant variables (U_1, U_2), variability was found. Needle length (1) was the trait with the greatest influence on U_1. Needle width (3), needle height (4) and distance from resin canals to the needle edge had the greatest influence on U_2.

Low differentiation among the trees in the analyzed population was confirmed by the calculated Mahalanobis distances. Only one pair of trees (tree nos. 9, 16) was highly differentiated ($\alpha = 0.01$) ($T^2_{calc.} > T^2_{crit.}$).

On the basis of Mahalanobis distances, the minimum spanning tree was drawn. Additionally, every Mahalanobis distance between two trees was examined by Hotelling T^2 statistics. The minimum spanning tree does not show any distance that differed significantly, that is, no pair of trees forming the minimum spanning tree significantly differed in respect to the analyzed needle traits. The minimum spanning tree for the discussed population is shown in Figure 4.

In the dendrogram (Fig. 5) constructed on the basis of agglomerative grouping by nearest-neighbor using the Euclidean distances, no group could be distinguished. This diagram confirmed the very low differentiation of trees.

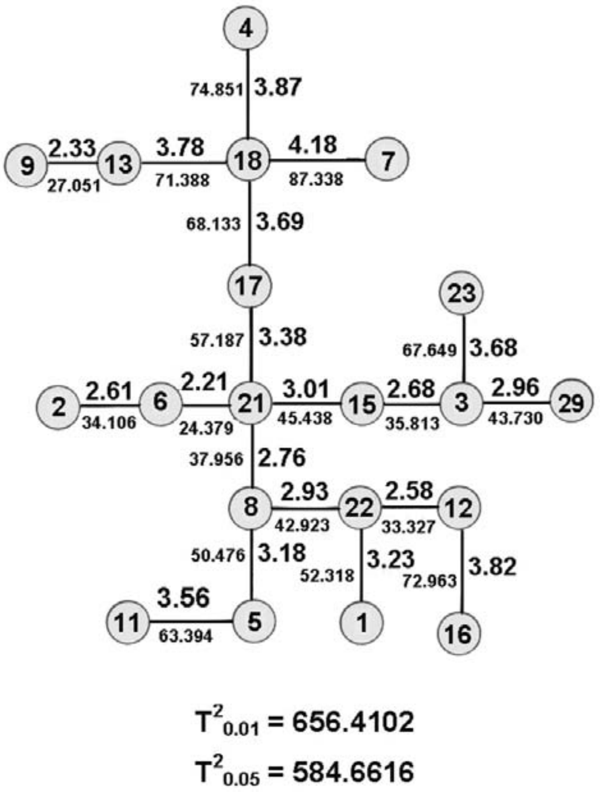

Fig. 4. Minimum spanning tree of 20 silver fir trees from the Tisovik Reserve population, constructed on the basis of shortest Mahalanobis distances (capital numbers) with Hotelling T^2 statistics (small numbers). $T^2_{0.05}$ and $T^2_{0.01}$ – critical value for Hotelling T^2 statistics. Mahalanobis distance is statistically significant if $T^2_{cal.} > T^2_{crit.}$.

DISCUSSION

This paper analyzed the intrapopulational variability of 12 needle traits of fir trees in the Tisovik Reserve. Research on fir needles generally is poorly developed. Previous studies on fir needles have concentrated mainly on differences caused by various environmental

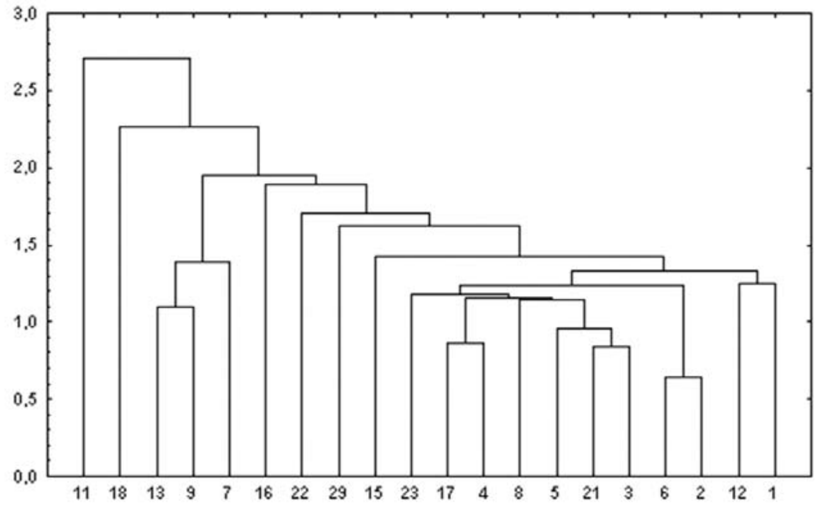

Fig. 5. Dendrogram of 20 silver fir trees from the Tisovik Reserve population, constructed on the basis of shortest Euclidean distances.

conditions at the locations where the trees were growing (Mańkowska, 1985). The needle structure of silver fir was described by Liu (1971) and Kausik and Bhattacharya (1977). Liu distinguished seven types of needle shape in cross sections, and stated that the location and diameter of resin canals are highly variable traits and as such are important traits for identification of *Abies* species and for general descriptions of silver fir needles.

There are several papers devoted to analysis of silver fir needle traits (Popnikola, 1974; Kormuták, 1985; Pawlaczyk et al., 2002), which differ in their research perspectives. Kormuták (1985) was interested in hybridization of *Abies* species, not in analyzing the inter- or intrapopulational variability of silver fir. In that study, parental species were compared to hybrids by several methods including needle traits. Popnikola (1974) made a comprehensive study of needle trait variability in Macedonia, and found that needle length decreased with increasing elevation. Differences between populations were observed especially in mechanical and assimilative tissues, as well as in resin canals. Generally the Macedonian populations were similar despite the noted differences.

In the present work, only one pair of trees from the Tisovik population was found to differ significantly in respect to the analyzed needle traits. Generally the studied population was characterized by minimal variability. This finding was confirmed by the dendrogram, the minimum spanning tree, and discriminant analysis. The individuals do not form groups on the diagrams, indicating that the population is not differentiated.

The same methods were used to analyze the variability of three Polish populations of *Abies alba*; one from artificial stands in the Białowieża Primeval Forest, and two from natural stands in the Kamienna Góra Reserve (Roztoczański National Forest) and the Łabowiec Reserve (Beskidy Mts) (Pawlaczyk et al., 2002). A comparison of the results from that study and the present one reveals that the silver fir population from Tisovik is characterized by the least variability of all these populations. The individuals from artificial stands in Białowieża and a natural stand in Łabowiec tend to group, whereas the trees from Tisovik and Kamienna Góra are more homogenous. This is especially visible in the graph of discriminant variables and in the dendrogram constructed on the basis of shortest Mahalanobis and Euclidean distances.

Mejnartowicz (1996) obtained a similar result. He compared the Cisovka population to eight others: four artificial stands in the Białowieża Primeval Forest, the Komańcza population (Bieszczady Mts), Tomaszów Lubelski (northeast Poland), Skarżysko (Świętokrzyskie Mts) and the Międzygórze population (Sudety Mts). The genetic diversity of silver fir from Cisovka was characterized by much lower average numbers of alleles per locus, and a much lower percentage of polymorphic loci than those populations. The Cisovka population had a significant excess of heterozygotes (H_{ob} = 0.123 and H_e = 0.079).

According to Mejnartowicz (1996), excess heterozygosity is the result of long selection for adaptation to rapidly changing environmental factors in the Białowieża Primeval Forest after the last glacial period. Heterozygous trees are expected to be more adaptable because natural selection reduces the frequency of homozygotes in natural forest populations (Stern and Roche, 1974).

Analysis of the intrapopulational structure of the Tisovik population, expressed in needle traits, confirmed the following:

1) calculated Mahalanobis distances indicated that only one pair of trees is significantly differentiated,
2) Student-t tests showed that the trait that best differentiated the studied individuals was needle length, and the trait that least differentiated the trees was the hypodermic cell width/height ratio,
3) discriminant analysis confirmed that the studied population is characterized by low intrapopulational differentiation,
4) the dendrogram constructed on the basis of shortest Euclidean distances showed that individuals did not form groups within the population.

On the basis of these results, it may be concluded that the Tisovik Reserve population is weakly differentiated. This finding points to the natural origin of European silver fir in the Białowieża Primeval Forest.

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