Allozyme Polymorphism in Seeds Collected from a IUFRO-68 Douglas-fir Test-Plantation

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Summary

The allozyme polymorphisms in wind-pollinated seeds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.) from a IUFRO-68 test-plantation was estimated. The seeds were analyzed as a pooled sample from all cone bearing trees originated of 41 populations, from a total of 71 individual trees at an age of 23 years, and compared with the parental population studied as individual trees.

Twenty-two loci of 13 enzyme systems were analyzed in the megagametophyte of the parental population. Allozyme variability levels were high. Seventeen of the 22 loci were polymorphic (P = 77,3 % at 0.99 criterion), with mean number of alleles per locus M/L = 2.68, effective number of alleles, Ne = 1.25. For polymorphic loci M/L = 2.94, and Ne = 1.31. Wright's fixation index for studied loci F = —0.007. The average expected heterozygosity, based on all investigated 22 loci was He = 0.192. Observed heterozygosity Hob = 0.191.

In the filial (embryos) population 6 polymorphic loci were investigated. For these He and Hob was identical, and amounted: 0.267. Fixation index F = 0.005 differ from this index, calculated for similar loci in maternal population where it was —0.082. The calculated multilocus outcrossing rate (tm = 0.966) was similar to those reported for natural stands of Douglas-fir.

From the results obtained one can suggest that seeds produced on this IUFRO-68 test plantation retained high level of genetic variation and low level of selfing. Previous quantitative works in this test plantation and presented results showed that seeds collected from the best growing provenances IUFRO-68 field experiment can serve for local reforestation purposes.

Key words: *Pseudotsuga menziesii*, Douglas-fir test plantation IUFRO, isoenzyme, genetic variation, outcrossing rate, heterozygosity.

FDC: 165.3; 165.5; 232.311.3; 174.1 *Pseudotsuga menziesii*.

Introduction

In a country were Douglas-fir is an introduced forest tree species, open pollinated seeds are often collected for commercial purposes, mostly from small experimental areas or even from small stands of unknown origin.

In 1966/1967 IUFRO section No. 22 undertook effort to establish a series of field experiments with West-American forest trees. For Douglas-fir in 30 countries more than 50 field experiments were established in 1968 to 1970 (Barner, 1973). One of them is located in Kórnik, and managed by the Institute of Dendrology.

Now trees on all of these experiments are 25 years old and more or less start to bear seeds, which could be collected and used for experimental purpose in many European countries. Such synthetic seed collections originated from a relatively small number of fructifying trees could carry a high level of selfed seeds, and then manifest inbreeding depression effects. However acting against inbreeding should be the great genetic diversity existing among sampled populations, originating from the huge area of Douglas-fir distribution.

The purpose of this paper was to determine the genetic variation and genetic diversity in seeds sampled of every tree bearing cones in IUFRO-68 Kórnik test-plantation of Douglas-fir.

Materials and Methods

Plant material

IUFRO numbers of the provenances are 1001 to 1104. Seeds were collected in natural stands from at least 15 dominating trees per population in British Columbia, Washington and Oregon. To avoid a possible effect of inbreeding, the space between the trees was approximately 100 m (Barner,, 1973). In 1968 seeds of 104 provenances of Douglas-fir were sown, without stratification, in 4 replications in the nursery at Kórnik. Of the initial pool of 104 populations, 4 populations were excluded from the experiments (Mejnartowicz, 1973) because of frost damages.

In 1970 3 year old seedlings (1 + 2) were planted on plant-free former arable land. Geographical localization of 4 hectare experiment is lat. 52° 15', long. 17° 06', altitude 70 m.

In 6 randomized blocs, there were 25 specimens of 1 population on 1 plot, and they were distributed in a 1.5 m x 2 m spacing. In between the rows being 2 m apart, black alder was planted. After 8 years the alder were cut down in order to receive a pure Douglas-fir stand. At the same time the first trees started to produce cones, and at 9 years age 69 % population possessed from 1 to 30 trees with cones (Mejnartowicz, 1976).

First thinning was made at age 20 years (1987). Afterwards it came to the first abundant cone crop and also 3 years later in 1990.

Cone samples were collected in 1990 from all trees with heavy cone crop, it is 71 trees (*Table 1*). We collected 8 litre cones per tree from apical, central and lower zone. El-Kassaby and Sziklay (1983), have shown that 40 to 60 individuals is needed to obtain reliable estimates of allelic frequencies on the population level in Douglas-fir stand.

Electrophoretical methods

The genotypes of 71 maternal trees were identified using the megagametophyte tissue for 13 enzyme systems (Enzyme Commission number, locus abbreviations and buffer systems upon which they were run, in parenthesis): esterase (EC 3.1.1.1, EstA, I), fluorescent esterase (EC 3.1.1.2, FleA, I) formate dehydrogenase (EC 1.2.1.2, Fdh, I) glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6pdh, I), glutamate dehydrogenase (EC 1.4.1.2, Gdh, I), glutamate oxalacetate transaminase (EC 2.6.11., GotA, GotB, GotC, I), isocitrate dehydrogenase (EC 1.1.1.42, Idh, II), menandione

Table 1. — List of Douglas-fir seed samples collected in 1990 on the Kórnik field experiment.

IUFRO no.	Population name	State	Lat.	Lon.	Alt m	No. of trees
1002	Dean	B.C.	52°48'	126°58'	6	2
1003	Alexandria	B.C.	52°42'	122°26'	640	1
1004	Stuie	B.C.	52°22'	126°00'	225	2
1005	Williams Lake	B.C.	52°07'	122°00'	600	1
1007	Clearwater	B.C.	51°39'	120°00'	450	2
1009	Klina Klini	B.C.	51°14'	125°35'	600	1
1012	Klina Klini	B.C.	51°07'	125°36'	3	2
1013	Revelstocke	B.C.	51°00'	118°12'	600	4
1022	Fly Hill	B.C.	50°32'	119°24'	750	1
1024	Owl Kreek	B.C.	50°20'	122°44'	210	1
1026	Stella Lake	B.C.	50°17'	125°28'	150	1
1027	Alta	B.C.	50°12'	122°52'	630	1
1030	Squamish	B.C.	49°17'	123°09'	15	2
1031	Gold River	B.C.	49°45'	126°04'	90	1
1036	Alberni	B.C.	49°20'	124°51'	135	1
1038	Chilliwack	B.C.	49°06'	121°42'	900	2
1039	Chilliwack	B.C.	49°04'	121°48'	165	3
1049	Backon Point	W.	48°36'	121°23'	495	1
1050	Marblemount	W.	48°35'	121°24'	120	3
1051	Dedro Woolley	W.	48°32'	122°19'	60	1
1052	Twisp	W.	48°23'	120°24'	780	2
1055	Newport	W.	48°12'	117°03'	720	1
1057	Granite Falls	W.	48°05'	122°02'	90	2
1058	Lake Crescent	W.	48°04'	124°00'	300	1
1059	Perry Creek	W.	48°03'	121°28'	600	1
1062	Forks	W.	47°59'	124°24'	90	2
1064	Hoh River	W.	47°48'	123°58'	240	1
1068	Chiwaukum	W.	47°41'	120°44'	540	2
1069	North Bend	W.	47°28'	121°45'	150	1
1075	Enumclaw	W.	47°16'	121°56'	240	2
1081	Alder Lake	W.	46°48'	122°17'	420	2
1085	Randle	W.	46°33'	122°03'	330	1
1090	Cougar	W.	46°05'	122°18'	495	2
1091	Yale	W.	46°00'	122°22'	120	2
1092	Glenwood	W.	46°00'	121°10'	480	1
1093	Willard	W.	45°48'	121°41'	495	7
1095	Prindle	W.	45°37'	122°08'	450	2
1100	Grand Ronde Age.	O.	45°06'	123°36'	180	2
1101	Waldport	O.	44°24'	123°52'	60	1
1102	Upper Soda	O.	46°23'	122°12'	975	2
1104	Brookings	O.	42°07'	124°12'	300	1

reductase (E. C. 1.6.99.2, MnrB, MnrC, II), phosphoglucose isomerase (EC 5.3.1.9, PgiA, PgiB, I) phosphoglucomutase (EC 2.7.5.1, PgmA, PgmB, I), sorbitol dehydrogenase (EC 1.1.1.14, Srdh, I), superoxide dismutase (EC 1.15·1.1, SodA, SodB, II).

According to sampling strategies recommend by Morris and Spieth (1978) 7 megagametophytes from each of 71 studied trees were analyzed to define genotypes. Megagametophyte tissue and embryo were isolated separately from the dry seeds and homogenized in 40 μl of Tris-HCl buffer pH 7.2 with the addition a 0.15 % p-mercaptoethanol.

Homogenates were subjected to horizontal starch gel electrophoresis by applying the two buffer systems (electrode/gel buffer): System I, 0.06 M lithium hydroxide- 0.3 M boric acid, pH 8.1/0.03M Tris — 0.005M citric acid — 1% electrode buffer, pH 8.5 (Ridgeway et al., 1970). System II, 0.13 M Tris- 0.043 M citric acid, pH 7.0/1:10 dilution of electrode buffer (Siciliano and Shaw, 1976).

Gel slices were stained for the activity of 13 different enzymes using recipes described by Tsay and Taylor (1984) for Fdh and for the other enzymes by Cheliak and Pitel (1984).

The progeny (embryos) was identified at six polymorphic loci: Fdh, G6pdh, GotB, Idh, MdhC, and SodA. At least 12 embryo-megagametophyte pairs were analyzed for each tree so the observed pollen allele contributed to each embryo were also determined. Inheritance of allozyme variants for investigated loci was confirmed from segregation ratios of haploid megagametophytes from heterozygous individuals (El-Kassaby et al., 1982; Adams et al., 1990; Lewandowski and Mejnartowicz, (1992). If more than 1 locus was resolved for a particular enzyme system, than most anodal was designated as locus A, the next as

B, etc. A similar procedure was employed for numbering allelic variants at each locus. Alleles lacking stain activity were designated as null (N).

Statistical analysis

Five single-locus measures of genetic variation were calculated. These were the average number of alleles per locus (M/L), effective number of alleles (Ne), the percentage of polymorphic loci (P) at which the frequency of the most common allele is less than 0.99, the observed (actual) heterozygosity (Ho), and the expected heterozygosity (He). The observed genotypic distribution in individual loci were analyzed for goodness of fit to HARDY-WEINBERG equilibrium by means of a G-test (SOKAL and ROHLF, 1973). The average effective number of alleles in mature stand was calculated after GREGORIUS (1987) as harmonic mean of the n values for individual loci.

Single-locus and multilocus rates of outcrossing were calculated based on the maximum-likelihood procedure of RITLAND and EL-KASSABY (1985) that were developed for conifers using an MLTF computer program.

WRIGHT's fixation index (F) was estimated to measure the proportional extent of inbreeding using formula: F = 1—Ho/He. When self-fertilization is the sole factor affecting level of inbreeding an expected equilibrium coefficient can be calculated: Fe = (1 — tm)/(1 + tm), where: tm is the outcrossing rate (ALLARD et al., 1968).

Results

Parental population

Twenty two loci were analyzed in this investigation (*Table 2*). Of these 22 loci, 5 (Gdh, MnrC, PgiA, SodB and Srdh) were monomorphic (at 0.99 criterion) in our material. The remaining 17 loci were polymorphic (P= 77 %), showing the presence of 2 to 5 allele in EstA and Fdh. Effective number of alleles for this 2 loci was lower and amounted Ne = 3.96 for EstA and only 1.47 for Fdh.

Average number of alleles per locus M/L was 2.68 for all loci and 2.94 for only the variable loci. Mean Ne was much lower than M/L and amounted only 1.25 of allele per locus and 1.31 for the variable loci.

Expected heterozygosity values ranged from 0.0 for monomorphic loci Gdh, SodB and Srdh to 0.748 for the 5-allelic locus EstA and 0.510 for 3-allelic G6pdh. The average expected heterozygosity (He) amounted 0.192. Observed heterozygosity (Hob) was almost identical to He and amounted 0.191 (*Table 2*).

Only slight excess of heterozygosity was obsereved in parental population and average WRIGHT's fixation index was —0.007.

Filial (embryos) population

Six only variable loci were surveyed in the embryos population. It was Fdh, GotB, MdhC, Idh, G6pdh, SodA. Three alleles were observed in each of GotB, MdhC, and and SodA and 4 alleles were observed in Idh and G6pdh. Five alleles were found in Fdh (*Table 3*). Compared to the maternal population additional alleles MdhC-3 and G6pdh-4 were revealed in embryos. These 2 alleles occurred however with low frequency 0.01 of MdhC-3 and 0.04 of G6pdh-4.

Observed heterozygosity did not differ from the expected and amounted to 0.267. A very small deficiency of heterozygotes was observed in the filial populations and average Wrights fixation index was calculated to be 0.005. However, negative F values for Fdh, Mdh3 and Idh indicate an ex-

Table 2. — The genetic variation within mature stand of Douglas fir. Enzyme systems assayed in megagametophytos, number of alleles per locus (M/L), effective number of alleles (Ne), expected heterozygosity (He), WRIGHT's fixation index (F), G-test for HARDY-WEINBERG equilibrium (G).

Enzyme systems (E.C.number)	Loci	No.of alleles	Ne	He	Hob.	F	G
Est (3.1.1.1)	-A	5	3.96	0.748	0.732	0.020	24.5*
Fdh (1.2.1.2)		5	1.47	0.320	0.352	-0.101	2.6
FEst(3.1.1.2)	-B	3	1.03	0.028	0.028	0	2.9
Gdh (1.4.1.3)		1	1	0	0	-	-
Got (2.6.1.1)	-A	3	1.03	0.028	0.028	0	2.9
	-B	3	1.36	0.266	0.296	-0.113	4.5
	-C	3	1.07	0.068	0.070	-0.029	0.2
G6pdh(1.1.1.49)		3	2.04	0.510	0.563	-0.104	1.7
Idh (1.1.1.42)		3	1.29	0.227	0.225	0.009	1.1
Mdh (1.1.1.37)	-A	2	1.06	0.055	0.056	-0.018	0.1
	-B	2	1.09	0.081	0.085	-0.049	0.3
	-C	2	1.20	0.166	0.183	-0.102	1.3
	-D	3	1.34	0.254	0.211	0.169	2.3
Mnr (1.6.99.2)	-B	3	1.99	0.498	0.465	0.066	2.0
	-C	2	1.01	0.014	0.014	0	0
Pgi (5.3.1.9)	-A	2	1.01	0.014	0.014	0	0
	-B	2	1.67	0.143	0.155	-0.084	0.9
Pgm (2.7.5.1)	-A	3	1.50	0.336	0.310	0.077	5.2
	-B	4	1.44	0.305	0.239	0.216	8.2
Sod (1.15.1.1)	-A	3	1.18	0.156	0.169	-0.083	1.1
	-B	1	1	0	0	-	-
Srdh(1.1.1.14)		1	1	0	-	-	-
Mean	M/L=	2.68	1.25	0.192	0.191	-0.007	-
Mean polymor. loci		2.94	1.31	0.222	0.221		

Table 3. — Comparison of genetic characteristics of the filial (embryos) population to the mother stand of Douglas fir. Ne- effective number of alleles, He- expected heterozygosity, He- observed heterozygosity F- WRIGHT's fixation index, G- test for HARDY-WEINBERG equilibrium (G).

Enzyme systems Loci (E.C.number)	No.of alleles	Ne	He	Hob	F	G
		embryos population				
Fdh (1.2.1.2)	5	1.24	0.325	0.354	-0.089	34.2**
Got (2.6.1.1) -B	3	1.29	0.224	0.212	0.054	3.5
Mdh (1.1.1.37) -C	3	1.22	0.178	0.181	-0.017	0.4
Idh (1.1.1.42)	4	1.27	0.211	0.213	-0.010	3.5
Gépdh(1.1.1.49)	4	2.10	0.523	0.493	0.057	5.5
Sod (1.15.1.1) -A	3	1.17	0.144	0.149	0.035	1.7
Mean	3.67	1.33	0.267	0.267	0.005	-
		mathernal population				
Fdh (1.2.1.2)	5	1.47	0.320	0.352	-0.101	2.6
Got (2.6.1.1) -B	3	1.36	0.266	0.296	-0.113	4.5
Mdh (1.1.1.37) -C	2	1.20	0.166	0.183	-0.102	1.3
Idh (1.1.1.42)	3	1.29	0.227	0.225	0.009	1.1
Gépdh(1.1.1.49)	3	2.04	0.510	0.563	-0.104	1.7
Sod (1.15.1.1) -A	3	1.18	0.156	0.169	-0.083	1.1
Mean	3.17	1.38	0.274	0.298	-0.082	-

***) Significantly differ at P = 0.01

cess of heterozygotes. The F value appeared to be statistically significant for the Fdh only. The F index calculated for the same subset of loci in mother population (F= —0.082), revealed some excess of heterozygosity (*Table 3*).

Mating system estimation was made on the results of an analysis of the same variable six loci in embryos and megagametophytes.

Single-locus (ts) estimates of outcrossing rate ranged from 0.878 for locus G6pdh to 0.994 for locus MdhC, with a minimum variance mean of 0.991, and were not significantly heterogeneous over the studied loci (*Table 4*). The multilocus (tm) estimate of outcrossing was 0.966 and was lower than the minimum variance mean of single locus estimates.

Discussion

Genetic variation and diversity parameters (i.e.: average expected and observed heterozygosity, proportion of polymorphic loci, average number and effective number of alleles per locus) were little higher compared to observation in natural Douglas-fir stands (YEH and O'MALLEY, 1980; MERKLE and ADAMS, 1987; LI and ADAMS, 1989; MORAN and ADAMS, 1989). This difference may be attributable primarily to origin of experimental stand, compressing gene pool of 100 populations spreads over huge area of British Columbia, Washington and Oregon. In such man-made stand is avoided family structure and small probability of mating among relatives. Some differences could be connected to the number and choice of loci involved and the sample sizes.

Table 4. — Single-locus (t_s) and multilocus (t_m) estimates of outcrossing rates and allelic frequencies in the outcrossed pollen pool in the studied popuation

locus	ts	Pollen pool A1	A2	A3
Fdh	0.980 (0.034)	0.033[a]	0.789	0.178[b]
GotB	0.989 (0.050)	0.094	0.997	0.009
G6pdh	0.878 (0.053)	0.456	0.503	0.041
Idh	0.957 (0.043)	0.080	0.896	0.023[c]
MdhC	0.994 (0.023)	0.908	0.091	0.001
SodA	0.900 (0.056)	0.923	0.005	0.072
t_s	0.991[d]			
t_m	0.966 (0.017)		Fe = 0.017[e]	

a) synthetic allele (1+3+5)
b) allel 4
c) syntetic allele (3+4)
d) minimum variance mean over loci
e) WRIGHT's fixation index under mating system equilibrium

The observed high level of genetic polymorphism (effective number of alleles for polymorphic loci amounted Ne = 1.31) and heterozygosity (He = 0.192 and Hob = 0.191) indicated that there was no loss of genetic riches in artificial population, however only 71 trees were analyzed. General quite high level of multilocus heterozygosity may be important for survival over long lifetime of trees (GREGORIUS, 1986), what is in agreement with the low mortality of seedlings and young trees in this experimental stand. Of the original planting of 9475 seedlings 99 % were still alive at age 7, and the cause of early field mortality was in 60 % of cases root damage caused by cockchafer grubs (MEJNARTOWICZ, 1976).

The slight excess of heterozygotes reported in the parent population has been observed in both natural and experimental populations of conifers (SZMIDT and MUONA, 1985; YEH et al., 1986; EL-KASSABY et al., 1987; LEWANDOWSKI et al., 1991).

Mating systems of trees is probably frequent not random and can by itself generate stable polymorphisms without any heterozygote selective prevailing (GREGORIUS and ZIEHE, 1986). For Douglas-fir BONGARTEN et al. (1985) stated that selection was not effective for enzyme locus heterozygosity.

Analysis of mating systems is based on so called the mixed-mating model, assumes all offspring are the result of either selfing or random outcrossing and that no selection occurs between germination and the census of seed progenies (BROWN et al., 1985). MÜLLER-STARCK and GREGORIUS (1988) criticized this model and shown that the 3 investigated by them loci were related to 3 distinct mating systems.

Comparison of single-locus and multilocus population estimates allows inference about the amount of inbreeding other than selfing (RITLAND and JAIN, 1981). Single locus estimates of outcrossing rate are known to be lowered by any form of inbreeding in addition to selfing, such as mating among relatives due to family substructuring of the population (SHAW and ALLARD, 1982). This has often been observed for natural populations (NEALE and ADAMS, 1985; EL-KASSABY et al., 1987. KNOWLES et al., 1987; YEH and MORGAN, 1987). However, substructuring is not possible in test plantation with randomly planting trees into population plot and randomized blocks of populations, where after thinning was only 15 trees of 1 population per plot. In this study the minimum variance mean of single-locus estimates (ts = 0.991) is greater than the multilocus one (tm = 0.966). It also suggest lack of mating among relatives. This has often been observed for artificial populations, such as seed orchards (BARRETT et al., 1987; MUONA and HARJU, 1989; BURCZYK, 1991).

The presented multilocus estimate of outcrossing is quite high (0.966) and very close to the multilocus estimates reported for a natural stand and a seed orchard of Douglas-fir (NEALE and ADAMS, 1985; RITLAND and EL-KASSABY, 1985; SHAW and ALLARD, 1982).

Small differences between F and Fe and lack of mating among relatives suggest that self-fertilization is the main factor affecting inbreeding in the studied population.

Results obtained in this study and in previous quantitative works (MEJNARTOWICZ, 1973 and 1976) suggest that seeds collected on a sufficiently large number of trees in the IUFRO-68 plot experiment in Kórnik, represent sufficiently diversified material for a breeding program aimed at expansion of Douglas-fir use in Poland.

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The Structure of the Pinus sylvestris L. in the Insular Pine Forests of the South Russian Plain

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Summary

Some morpho-anatomical traits of cones, seeds, pine-needles and biochemical traits (isoenzymes, essential oils) of *Pinus sylvestris* L. were studied with the help of factor analysis in disjunct forests of the south Russian plain.

It is found out that 56% of the variation is accounted for by the first factor, which is connected with isoenzymes.

The second factor accounts for 22% of the variation and is determined by needle length (weight coefficient 0.882), filled seeds (0.849) and by cone size, with diameter weight coefficient 0.793 and length, 0.728.

The third factor accounts for 9% of variation and is linked to the monoterpene fraction of the essential oils.

The borders of defined populations do not coincide with forest type boundaries but approach the eco-climatic zones of the Russian plain.

Key words: Population, insular pine forests, *Pinus sylvestris*, factor analysis.

FDC: 165.5; 174.7 *Pinus sylvestris*; (470).

Introduction

Pinus sylvestris L. growing in different ecological and geographical conditions has formed a great quantity of forms, ecotypes and subspecies in the process of evolution.

The variability of different traits is particularly high in island and relict pine forests of the south Russian plain (PRAVDIN, 1964; LARIONOVA, MILUTINA et al., 1988).

It is evident from the literature on this species that the search for morpho-physiological (PRAVDIN, 1964; MAMAEV, 1973; CHEREPNIN, 1980; MAMAEV and MAKHNEV, 1982, 1988; SIDELNIKOVA and MURATOVA, 1991) and biochemical traits (ALTUKOV, KRUTOVSKY, DUKHAREV et al., 1989; CHERNODUBOV and DERYUZKIN, 1990; GONCHARENKO, PADUTOV, SILIN et al., 1991) which would reflect the genetic structure of natural stands has been carried out intensively.

Some authers (VIDYAKIN, 1991a and b; YABLOKOV, 1980) assert that the most informative method involves indices.

Some researchers (KRAVTSOV and MILUTIN, 1981, 1985; SEMERIKOV, 1981, 1986; MILUTIN, 1982) consider that success is achieved only when studying a complex of traits and making use of multivariate analysis.

The purpose of this work is to select the most informative traits of some morpho-anatomical and biochemical indices in these pine forests for studying population structure.

Material and Methods

The chalk (calciphilic) "island" pine forests of the Privolzhskoy, the middle-Russian hills, the Donetsk ridge and sandy (acidophilic) pine forests of the Russian plain were the subject of the investigation (*Table 1*).

All these stands are native forests and they are protected areas (forest reserves, game reserves, genetic reserves, monuments of nature, etc.). Sample plots were laid out in these areas. Then 25 to 50 trees were randomly selected in each stand with the exception of the stand in Novo-Oskol where only 11 trees remained. In October-November, 1987, collections were made from the same trees, from the southern side of the middle part of the crown of 30 cones and samples of needles; part of these were fixed in 96% ethyl alcohol and glycerin in 3:1 mixture, and stored for studying morpho-anatomical traits. The other portion of needles in polyethylene bags was kept in a refrigerator for extraction of essential oils.

The length of 20 pairs of needles from every tree was measured by means of a ruler. The width and thickness on 10 microscopic cross-sections of every needle were determined by means of an eyepiece micrometer. The same preparations were used to count the number of resin canals (PRAVDIN, 1964; MAMAEV, 1973).