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Changes Induced by Zinic Smelter Pollution in the Genetic Structure of Pine (Pinus sylvestris L.) Seedling Populations

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Summary

Comparison of the genetic structure of a seedling population grown in an unpolluted area and in an area adjacent to a zinc smelter has demonstrated differences. When seedlings of *Pinus sylvestris* were grown in the polluted area both heterozygosity and degree of genetic polymorphism underwent a significant decrease (by 30% and 20%, respectively) compared to from the control in the unpolluted area. For studied enzymatic loci the seedling population grown on the polluted site failed to maintain HARDY-WEINBERG equilibrium, exhibiting an excess of homozygotes compared to expected levels. The changes in genetic parameters point to directional selective processes in the studied seedlings groups.

Key words: zinc pollution, genetic structure, seedlings, isozymes, Scots pine.

Introduction

Studies on the genetic structure of naturally regenerating populations in polluted regions have demonstrated more extensive changes than in populations free from pollution (PRUS-GLOWACKI and NOWAK-BZOWY, 1989). Studies on the adaptive strategy in naturally regenerating populations suffer the disadvantage that precise determination of the genetic structure of seed pools, serving as starting material for regeneration is difficult and involves some approximation. This is because in different years different individuals in the population variously participate in seed set and in the composition of the pollen cloud, and thus the gene pool of the embryo population changes each year. Also only general conclusions can be drawn from studies performed on changes in the genetic structure in two distinct populations, originating in either heavily or only slightly polluted regions and therefore representing different gene pools.

This situation induced us to undertake studies on changes in the genetic structure of pine seedling populations, using a single pool of seeds of known genetic structure for the establishment of a field experiment. Comparison of allelic and genotypic frequencies at defined time periods in the embryo population and in populations of surviving seedlings may demonstrate whether the increased mortality, observed in the polluted areas compared to controls, represents the result of a stochastic process or a directional change in the genetic structure of the population associated with selection. The need for these kind of studies is strongly recommended to reduce our gaps in knowledge, of the quantifying of genetic losses caused by anthropopressure (KARNOVSKY et al., 1989).

Material and Methods

Seeds collected from 100 trees of a pine population in Zielonka Forest, near Poznan, an area only slightly polluted by industry, served as the starting material to set up experimental plots. The choice of the seeds sown for experiments characterized as of "average" genetic structure (frequencies of genotypes) for a Scots pine population were not already changed by industrial pollution in the Upper Silesia region. From each of the 100 trees, 100 seeds were collected, pooled and distributed in portions of 3000 seeds each to be sown in the study areas. Before setting up experimental plots, the percentage of germinating seeds was established in the loboratory. Genetic structure of the seed pool was determined by isoenzyme analysis of germinating embryos.

A total of three experimental sites were set up. Two of them were located at distances of approximately 1000 m and 1500 m, respectively from a zinc smelter in Miasteczko Slaskie (Upper Silesia) while the third, a control, was located in Zielonka Forest near Poznan the site from which the seeds were collected. At the time of sowing, soil samples were taken from each area and analyzed for content of heavy metal ions.

The plots were set up in 1987, mid-April, the recommended time for sowing pine in this part of Poland. The seeds, 3000 per plot, were spot-sown at a depth of approximately 1 cm, spaced 5 cm in rows. The distance between seedlings is designed to restrict density selection. Care

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of the experimental plots involved periodic manual weeding.

Parallel with collecting the material for isoenzyme analyses the numbers of seedlings in the plots were estimated and the extent to which they were damaged was evaluated, using a four point scale. Notation 0 corresponded to absence of evident injuries, 1 denoted noticable injuries, 2 — marked injuries and 3 — dead seedlings. Because most of the plants died in the plots close to the zinc smelter, the seedlings were sampled for isoenzymatic analysis twice only, in November 1987 at age eight months and in April 1988 at age one year and the data were pooled together. In Spring 1989 (age two years) survival was below 3% and only 18 seedlings showed no injuries.

Isoenzyme analyses were than performed on seedlings with injury scores 0 to 2. However, due to low activity of some enzymes in the seedlings and the respective difficulties in interpretation of isoenzyme patterns, only five loci were analysed out of 11 studied in the embryo population. The enzyme loci examined are listed in *table 1*. Isoenzyme patterns in gels, staining and genetic interpretation correspond to those described by RUDIN and EKBERG (1978), SZMIDT and YAZDANI (1984) and GULLBERG et al. (1982).

Calculations of the following genetic parameters were made, in the studied groups of seedlings namely: heterozygosity (Ho and He), genotype polymorphism index (Pg), fixation index (F), genetic similarity indices based on gene (SN) and genotype (SH) frequencies and genetic distances (DN and DH).

These calculations were conducted as described by NEI and ROYCHOUDHURY (1974), JAIN and WORKMAN (1967), KAHLER et al. (1980), HEDRICK (1974), using the Gen computer program (BZOWY and NOWAK-BZOWY, 1988, unpublished).

Results

The levels in the soil of three main metals, apparently emitted by the zinc smelter (zinc, lead and cadmium)

Table 1. — Enzymatic loci investigated in the groups of studied individuals.

Enzymatic loci	E.C. No. and denotation of loci	Embryos No. of alleles	Seedlings No. of alleles
Glutamate dehydrogenase	1.4.1.3 GDH	2	2
Malate dehydrogenase	1.1.1.37 MDH A	2	2
	MDH C	2	- *
Shikimate dehydrogenase	1.1.1.14 ShDH A	6	5
	ShDH B	2	3
Alkohol dehydrogenase	1.1.1.1 ADH	2	- *
6 - phospho-gluconate dehydrogenase	1.1.1.49 6 PGD	3	- *
Glutamate-oxaloacetate transaminase	2.6.1.1 GOT A	3	- *
	GOT B	4	- *
Diaforase	1.6.99 Diaf	3	3
Fluorescent esterase	3.1.1.1 Fest	4	- *

*) — no satisfactory separation of enzymatic bands or lack of enzyme activity.

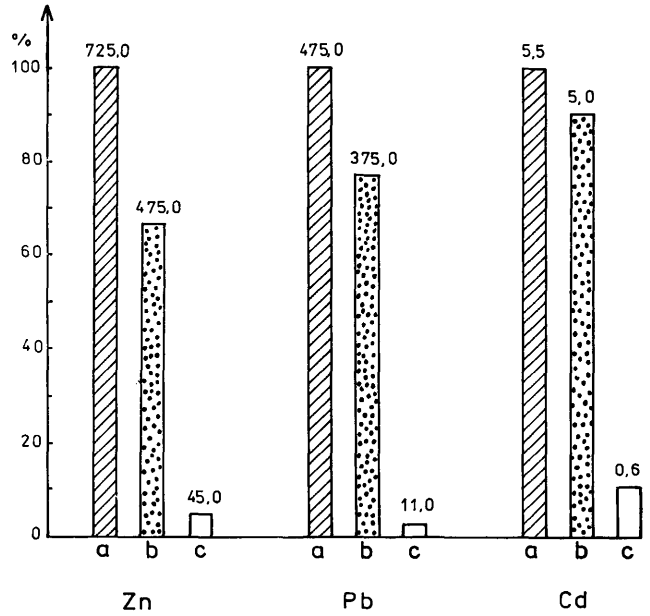

Figure 1. — Contents of three heavy metals in the soil samples from experimental sites (a and b — from Silesia; 1000 m and 1500 m from a zinc smelter, c — from Zielonka Forest). The values are in mg per 1 kg of soil.

are compared for two experimental plots at Miasteczko Slaskie and for the control in Zielonka Forest (*Figure 1*). The extent of air pollution in the region of the zinc smelter and in the Zielonka Forest is shown in *table 2*. As can be seen industrial pollution was several times more intense in Miasteczko Slaskie than in Zielonka Forest for all 10 pollutants (*Figure 1* and *Table 2*).

Survival of seedlings and extent of injuries to the plants in individual experimental plots are presented in *table 3*. In Zielonka Forest, 50% of the seeds germinated, as compared to 37% and 34% close to the zinc smelter and 78% in laboratory conditions. Analysis of the extent and quality of seedling injuries in the form of necrotic spots and discolorations, showed that only 11% of plants in

Table 2. — Concentration and deposition of major pollutants in Miasteczko Slaskie area (Upper Silesia) and in Zielonka Forest near Poznan in the growing season (16 April to 15 October) and in the heating season (16 October to 15 April) 1987/1988 (winter). Data from WSSE Katowice and WSSE Poznan.

Pollutant	Concentration ug/m^{-3} and season			
	growing season		heating season	
	Miasteczko Slaskie	Zielonka Forest	Miasteczko Slaskie	Zielonka Forest
SO_2	29.00	4.24	125.00	14.80
N_2O_5	74.00	13.60	88.00	36.00
F_2	1.40	0.036	1.10	0.050
NH_3	24.70	-	28.00	-
Aerosols	140.00	-	158.00	-
Pb	0.49	-	0.53	-
Zn	0.66	-	0.98	-
Cd	0.0153	-	0.0213	-
Pb *	518.40	0.00	478.80	0.00
Cd *	12.47	0.30	12.77	0.30
Zn *	1106.90	9.91	1199.70	8.15

*) deposition in $mg/m^{-2}/a^{-1}$

Table 3. — The results of field experiments showing the survival of *Pinus sylvestris* seedlings and the degree of injury. O — lack of visible traces of injury, 1 — changes in coloration of needles, 2 — necrotic spots on needles, 3 — dead seedlings (autumn 1988).

	Germination of seeds in laboratory %	No. of seeds	No. of seedlings	Percent of seedlings in classes			
				0	1	2	3
Zielonka Forest	78	3000	1475	98	0	0	2
1000m from smelter	78	3000	811	11	22	29	27
1500m from smelter	78	3000	909	11	26	38	25

Table 4. — Allelic frequencies for 5 loci in the groups of studied individuals.

Locus	Alleles	Embryos n=100	Zielonka n=184	Silesia n=168
GDH	1	0.620	0.625	0.651
	2	0.380	0.375	0.348
Diaf	1	0.865	0.885	0.866
	2	0.095	0.066	0.076
	3	0.040	0.053	0.057
	4	0.000	0.000	0.000
ShDH A	1	0.770	0.807	0.889
	2	0.125	0.119	0.079
	3	0.025	0.038	0.019
	4	0.005	0.005	0.000
	5	0.055	0.029	0.011
	6	0.020	0.000	0.000
ShDH B	1	0.940	0.940	0.944
	2	0.060	0.056	0.048
	3	0.000	0.002	0.006
MDH A	1	0.940	0.959	0.979
	2	0.060	0.040	0.020

the two polluted plots were free from visible injuries. As many as 25% to 27% of plants perished (in Zielonka Forest only 2%) while 62% and 64% of the remaining, showed visible injuries.

Allele and genotype frequencies in the groups of individuals compared have been presented in *table 4* and *table 5*. Some observed differences in allele frequencies, between the compared groups of individuals, particularly for GDH, ShDHA and MDHA loci failed to be confirmed by the chi-square test. On the other hand, in the case of genotypes, significant differences in frequencies of ShDHA11 and MDHA12 genotypes were observed between the embryo group and the group of seedlings from Silesia. The ShDHA11 genotype was markedly more frequent and MDHA12 more rare in the sample of seedlings from the polluted region. A similar tendency for differential frequency in the studied group of plants was observed for some other genotypes but the differences proved non — significant in the chi-square test. For example, this was so for the ShDHA12 genotype which was less frequent among seedlings from the zinc smelter region.

Number of alleles and genotypes observed for individual loci as well as the number of alleles per locus in the analysed groups of individuals have been presented in *table 6*. For ShDHA locus a lower number of genotypes was noted in the seedling sample from Silesa as compared to the population of embryos and of seedlings from Zielonka Forest. Also a slightly lower average number of alleles per locus was found in Silesia (3.0 and 2.8 respectively.

Table 5. — Genotypic frequencies for 5 loci in the groups of studied individuals.

	Genotypes	Embryos n=100	Zielonka n=184	Silesia n=168
GDH	1/1	0.390	0.423	0.470
	2/2	0.150	0.173	0.166
	1/2	0.460	0.402	0.363
Diaf	1/1	0.750	0.796	0.754
	2/2	0.020	0.010	0.005
	3/3	0.000	0.016	0.016
	1/2	0.150	0.101	0.142
	1/3	0.080	0.074	0.082
	1/4	0.000	0.000	0.000
ShDH A	1/1	0.620	0.657	0.819xx
	2/2	0.030	0.010	0.022
	3/3	0.000	0.005	0.000
	5/5	0.020	0.000	0.000
	6/6	0.010	0.000	0.000
	1/2	0.170	0.206	0.101
	1/3	0.050	0.043	0.033
	1/4	0.000	0.010	0.000
	1/5	0.060	0.038	0.005
	1/6	0.020	0.000	0.000
	2/3	0.000	0.005	0.000
	2/4	0.010	0.000	0.000
	2/5	0.010	0.005	0.011
	3/5	0.000	0.016	0.005
ShDH B	1/1	0.900	0.881	0.896
	2/2	0.020	0.000	0.006
	1/2	0.080	0.112	0.084
	1/3	0.000	0.005	0.013
	3/3	0.000	0.000	0.000
MDH A	1/1	0.880	0.919	0.959
	1/2	0.120	0.080	0.040xx

**) — statistically sigiificant differences in frequency in the studied groups of plants compared to the embryo population (chi-square test).

Table 6. — Number of alleles (A) and genotypes (G) in the studied group of individuals.
Zielonka Forest (unpolluted), Silesia (polluted). Average number of alleles per locus are given in brackets.

Locus	Embryos n=100		Zielonka Forest seedlings n=184		Silesia seedlings n=168	
	A	G	A	G	A	G
GDH	2	3	2	3	2	3
Diaf	3	4	3	5	3	5
ShDH A	6	10	5	10	4	7
ShDH B	2	3	3	3	3	4
MDH A	2	2	2	2	2	2
Together	15(3.0)	22	15(3.0)	23	14(2.8)	21

Data on heterozygosity (Ho and He), fixation index (F) and genotype polymorphism (Pg) in the studied groups of individuals is presented in *table 7*. The most extensive differences in heterozygosity level between the studied groups were noted for MDHA, ShDHA and GDH loci, and less pronounced ones for the remaining loci. In the majority of cases the observed heterozygosity (Ho) was lower in the seedling samples from Silesia ie. 6 versus 67%, in the embryo population and the population of seedlings from Zielonka Forest. The ShDHB locus represented an exception; both in Zielonka Forest and in Silesia the seedlings showed greater heterozygosity compared to the embryos.

Genotype polymorphism indices (Pg) were showing extensive differences between the studied groups of seedlings as compared to the population of embryos for ShDHA and MDHA loci. At the two sites the Pg index was lower in the seedling samples from Silesa 45% and 75% than in the population of seedlings from Zielonka

Table 7. — Heteroygosity observed (Ho), heterozygosity expected (He) fixation index (F) and genotypic polymorphic indices (Pg) in the studied groups of individuals.

Locus		Embryos	Zielonka Forest	Silesia
GDH	Ho	0.460	0.402	0.363
	He	0.471	0.468	0.453
	F	0.023	0.142	0.200
	Pg	0.613	0.628	0.619
Diaf	Ho	0.230	0.176	0.224
	He	0.241	0.209	0.240
	F	0.046	0.157	0.069
	Pg	0.408	0.348	0.404
ShDH A	Ho	0.320	0.326	0.154
	He	0.387	0.332	0.201
	F	0.174	0.017	0.214
	Pg	0.578	0.521	0.316
ShDH B	Ho	0.080	0.118	0.097
	He	0.112	0.111	0.104
	F	0.290	-0.060	0.071
	Pg	0.183	0.209	0.189
MDH A	Ho	0.120	0.080	0.040
	He	0.112	0.077	0.039
	F	-0.063	-0.041	-0.020
	Pg	0.211	0.147	0.078
m/loc	Ho	0.242	0.220	0.176
	He	0.265	0.239	0.208
	F	0.087	0.079	0.151
	Pg	0.399	0.371	0.321

Forest, this was 10% to 30% respectively lower than in the embryos. The mean value of the Pg index for studied loci decreased 20% in the population of seedlings from Silesia and about 8% in the seedlings from Zielonka Forest.

Wright's fixation index (F) which in the embryo group exhibited a slight excess of homozygotes compared to the proportion predicted by the Hardy-Weinberg equilibrium (F = 0.087) in the sample of seedlings from Zielonka Forest was approximateli 9% lower, probably due to elimination of individuals originating from selfing. In the

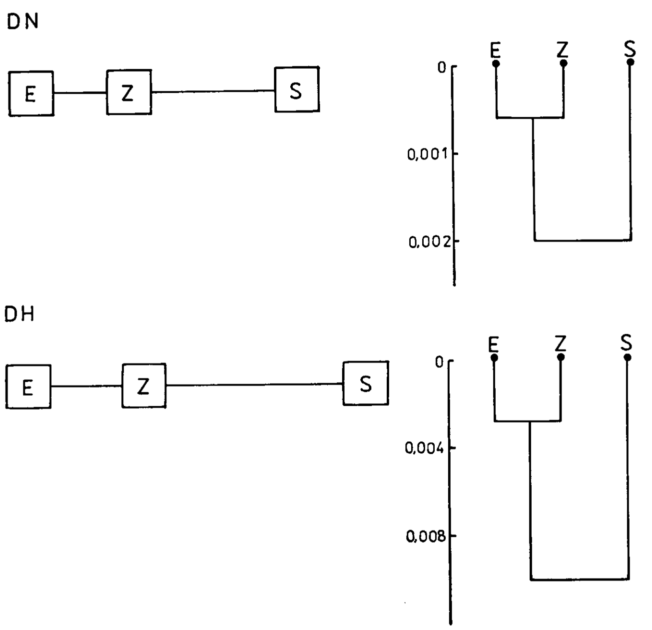

Figure 2. — Dendrites and dendrograms showing the genetic distances acc. to Nei (DN) and Hedrick (DH) between the studied groups of individuals.
E — population of embryos; Z — seedlings from Zielonka Forest; S — from Silesia.

seedling group from Silesia, the fixation index (F) pointed to a significant excess of homozygotes (F = 0.150), most pronounced in GDH (0.200) and ShDHA (0.214) loci. Indices of genetic similarity (*Figure 2*) between the three groups of individuals studied, based on allele and genotype frequencies showed that genetic structure of the seedling groups from Zielonka (Z) and from Silesia (S) differed decisively from that of the embryo population (E).

Discussion and Conclusions

Our studies on the impact of industrial pollution on *Pinus sylvestris* seedling populations have demonstrated that pollution significantly affects the gene pool and the genetic structure of surviving groups of trees. The effect has been expressed by genotype polymorphism levels which decreased approximately 20% and by average heterozygosity levels which decreased about 30% as compared to both the embryo population and the control population from the region of low level of industrial pollution.

Interestingly, the changes in the genetic structure of age groups of naturally regenerating pine populations are associated with increased heterozygosity level in the populations from the youngest to the oldest age classes (Prus-Glowacki and Nowak-Bzowy, 1989; Yazdani et al., 1985; Tigerstedt et al., 1982) while in our present studies up to 2 years old seedling populations have demonstrated a decreased genetic variability in this extremely polluted area. The observed differences might have resulted from the intense selection close to the zinc smelter, permitting survival of very few individuals manifesting appropriate genetic characters (*Table 4 and Table 5*). In the polluted region the most pronounced alterations are noted for the loci ShDH, MDH and GDH. While it evidently remains difficult to associate the studied enzyme loci directly with metabolic pathways conditioning tolerance or sensitivity of pine to pollution with a whole range of agents, these loci seem to be good markers in this type of study, even if they reflect only a small part of the pine genome. Results obtained so far should be regarded as preliminary. Longer experiments and larger number of observations should better reveal tendencies and directions of changes in the genetic structure of populations exposed to air and soil pollution. The studies will probably also permit a direct demonstration of an erosion of the population gene pool and reduction of genetic variability associated with industrial pollution.

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The Geographic Variation in European Silver Fir (Abies alba Mill.)

Gas Exchange and Needle Cast in Relation to Needle Age, Growth Rate, Dry Matter Partitioning and Wood Density by 15 Different Provenances at Age 6

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Abstract

Photosynthesis, respiration and transpiration were studied in one- and two-year-old needles of 15 *Abies alba* (MILL.) provenances at the age of 6 years. Needle cast and nutrient content were registered for the last four year's needles. Differences in all traits could be demonstrated. Especially, differences between provenances in the rate of senescence of the photosynthetic apparatus (needles) where obvious. Thereby provenances from central and eastern Europe showed pronounced decline in photosynthetic capacity and heavy needle losses over needle age compared with provenances from Calabria (southern Italy).

In addition height, diameter, wood density and dry matter partitioning were measured. Provenance differences in all traits could be demonstrated. Thereby seed sources from Calabria (southern Italy) were characterized by a high growth vigor compared with the provenances from central and southeast Europe.

Key words: Photosynthesis, respiration, transpiration, dry matter partitioning, growth, wood density, *Abies alba*, provenances, senescence, silver fir decline.

Summary

Photosynthesis, respiration and transpiration were studied in one- and two-year-old needles of 15 *Abies alba* (MILL.) provenances (respectively 6 from central Europe, 5 from southeastern Europe and 4 from southern Italy/Calabria) at the age of 6 years. In addition, needle cast and nutrient content were registered for the last four year's needles. Gas exchange was measured under controlled conditions (20 °C, 35% relative humidity, 480 $\mu E \cdot m^{-2} \cdot s^{-1}$) on small twigs by using a CO_2/H_2O-Porometer (Walz, Effeltrich/FRG). At the end of the sixth growing season the material was analysed for height and diameter growth, wood density and dry matter partitioning.

The results demonstrate significant differences between provenances in almost all traits measured, which mainly could be attributed to regions. Thereby provenances from southern Italy (Calabria) behaved quite differently compared to provenances from the rest of the natural distribution of the species. Differences between provenances from central and southeast Europe were mostly absent.

Photosynthesis of the one-year-old needles was slightly higher for the Calabrian provenances compared with the provenances from the other regions (central and southeastern Europe). For the two-year-old needles, a drastic reduction in photosynthesis was registered for the central and southeast European provenances. By contrast, the Calabrian provenances maintained high rates of photosynthesis. The respiration rates of the one-year-old needles were slightly lower for the Calabrian provenances in comparison to seed sources from the rest of the natural distribution. The two-year-old needles of the Calabrian provenances were characterized by higher rates of respiration.

The Calabrian provenances showed higher transpiration rates. Water use efficiency did not differ between provenances as far as the one-year-old needles are concerned. The two-year-old needles of the Calabrian provenances were characterized by a pronounced better water use efficiency (less units water transpired in order to fix one unit CO_2). This indicates overall better water relations and thereby a better adaptation to drought of the Calabrian provenances compared with seed sources from central and southeast Europe.

The big differences in needle cast between provenances could almost entirely be explained by regions. Thereby pronounced losses of older needles were registered in the central and southeast European provenances, whereas the Calabrian seed sources showed merely moderate losses.

The Calabrian provenances were characterized by high growth rates (height, diameter, dry matter and stem volume: respectively 19%, 13%, 45% and 47% above a mean of all provenances) and exhibited a pronounced

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