CHANGES OF GENETIC STRUCTURE OF *PINUS SYLVESTRIS* L. POPULATIONS EXPOSED TO INDUSTRIAL POLLUTION

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ABSTRACT

Isoenzymatic variation of *Pinus sylvestris* was analysed in populations from a provenance trial (IUFRO 1982) in Kórnik and their replication in Luboń. The two groups of populations originated from the same seed pool, but the one in Luboń was exposed to pollutants emitted during the production of nitrogen-phosphorus fertilizers,while the Kórnik provenance trial were used as a control group.

The parameters determining the genetic structure of the studied populations – frequency of alleles and genotypes (average number of alleles and genotypes per locus), observed heterozygosity (H_o), and genotypic polymorphism index (P_g) – have higher values in the group of trees tolerant to pollution. Also fixation index (F), which indicates that homozygotic genotypes underwent selection in this group, as well as dendrites and dendrograms according to Nei and Hedrick, suggest that the genetic structure of the populations exposed to pollution was changed.

The obtained results indicate that populations characterized by a greater genetic diversity are more tolerant to pollution.

KEY WORDS: *Pinus sylvestris*, provenances, genetic structure, air pollution tolerance

INTRODUCTION

The increasing industrialization and the pollution caused by it, have a considerable influence upon the vitality and survival of plants. Forest decline is a visible signal which shows the disturbance of the ecological equilibrium by industrial pollution. It was found that there exists intraspecific variation in woody plants tolerance to pollution. This phenomenon was observed also at the population level for *Pinus sylvestris* (Oleksyn, 1988).

Scots pine, *Pinus sylvestris*, is one of the major forest-forming species in Poland, which is particularly sensitive to industrial pollution (Białobok, 1984; Białobok et. al. 1984). Because of this, Scots pine populations are very good model for the study of microevolutionary processes taking place under the influence of man. Research on adaptive processes in populations of *Pinus sylvestris* showed that the ability of adaptation of this species to its environment is linked with the genetic structure of the population (Mejnartowicz, Palowski, 1989; Prus-Głowacki, Nowak-Bzowy, 1989). It was also found that isoenzymes may be used as markers linked with processes of selection caused by anthropogenic factors (Mejnartowicz, 1983, 1984).

In 1982 an IUFRO provenance trials was established at Kórnik and Luboń with the use of Scots pine seeds collected in 1978-1980 in eleven European countries, covering a considerable part of the European range of distribution of this species. The main purpose of this experiment was to study the morphological and physiological differences between the two groups of populations with their response to environment pollution. It was found that there exist statistically significant differences in the height growth of the trees, which were 43% lower in the area polluted with SO_2 and fluorides than in the control plots. Also a strong needle necrosis and a high death rate of trees was observed. In the populations exposed to pollution only about 50% of trees survived, as compared to 77% in control populations. In the contaminated area trees started to flower four years later, and the average percentage of trees producing flowers was three times smaller. Moreover, the juvenile period, i.e. from seed germination to the first flowering, is twice longer than in Kórnik (Oleksyn et al., 1992a). The above-mentioned authors found also that the response of pine trees to pollution depends on their origin. Provenances from the northern and southern parts of the European range of *Pinus sylvestris* are relatively tolerant to pollution as far as growth and needle necrosis are concerned, but are more sensitive in relation to flowering. Populations from Central Europe show the reverse tendency. Thus, the sensitivity of vegetative organs is not necessarily connected with the sensitivity of generative organs. These conclusions agree with an earlier report which suggested that the sensitivity of northern and southern populations is correlated with their lower productivity and efficiency of photosynthesis (Oleksyn 1988).

The aims of the present study were: (1) to investigate the genetic structure of *Pinus sylvestris* populations that were exposed to heavy pollution; and (2) to verify the hypothesis that

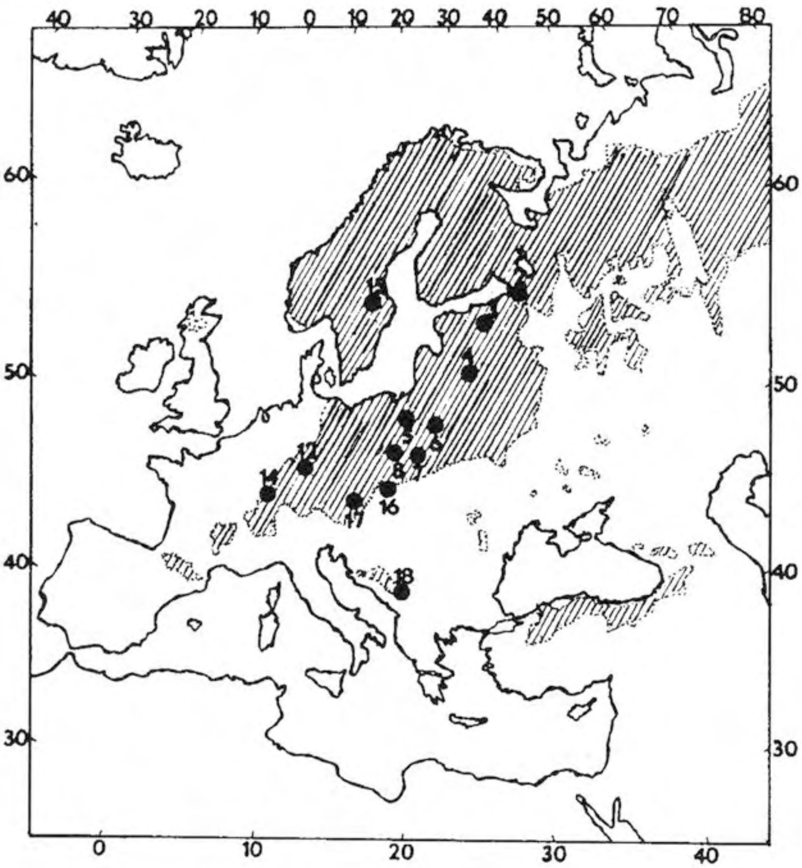

Fig.1 Geographical origin of the studied populations of *Pinus sylvestris* (after Oleksyn 1988):
2. Kondezhskoe, Russia (59°58N, 33°30E), 3. Serebryanskoe, Russia (59°50N, 29°07E), 4. Silene, Latvia (55°45N, 26°40E), 5. Miłomłyn, Poland (53°34N, 20°00E), 6. Supraśl, Poland (53°12N, 23°22E), 7. Spała, Poland (51°37N, 20°12E), 8. Rychtal, Poland (51°80N, 17°55E), 12. Lampertheim, Germany (50°00N, 10°00E), 14. Haguenau, France (48°49N, 7°47E), 15. Sumpberget, Sweden (60°11N, 15°52E), 16. Zahorie, Slovakia (48°46N, 17°03E), 17. Pornoapati, Hungary (47°20N, 16°28E), 18. Maocnica, former Jugoslavia (43°10N, 19°30E).

TABLE 1. The origin of the studied populations of *Pinus sylvestris.* N°K – number of studied individuals in populations from Kórnik (unpolluted area), N°L – number of studied individuals in populations from Luboń (polluted area).

Populations	**Country**	Lat. (N)	Long. (E)	N° of pop.	**N° K**	N° L
Kondezhskoe	Russia	59° 58'	33° 30'	2	30	30
Serebryanskoe	Russia	59° 50'	29° 07'	3	30	30
Silene	Latvia	55° 45'	26° 40'	4	30	30
Miłomłyn	Poland	53° 34'	20° 00'	5	30	30
Supraśl	Poland	53° 12'	23° 22'	6	30	30
Spała	Poland	51° 37'	20° 12'	7	30	30
Rychtal	Poland	51° 80'	17° 55'	8	29	30
Lampertheim	Germany	50° 00'	10° 00'	12	30	30
Haguenau	France	49° 49'	7° 47'	14	30	30
Sumpberget	Sweden	60° 11'	15° 52'	15	30	30
Zahorie	Slovakia	48° 46'	17° 03'	16	30	30
Pornoapati	Hungary	47° 20'	16° 28'	17	30	21
Maocnica	ex- Jugoslavia	43° 10'	19° 30'	18	30	30

the differences in the morphology and physiology of the trees growing under the selective pressure of pollution are somehow related to genetic structure of the population.

MATERIALS AND METHODS

Isoenzymatic variation of *Pinus sylvestris* was analysed from a provenance trial (IUFRO 1982) in Kórnik and Luboń originated from the same seeds pool. Geographical origin of the studied populations of *Pinus sylvestris* is shown in Fig. 1 and Tab. 1. The population in Luboń was exposed to pollutants emitted by Poznań phosphate fertiliser plant (PPFP). The field experiment was located about 2 km from the source of emissions. As a control for that area the experimental site was located 12 km southeast of PPFP in Kórnik , in a region relatively free of pollution.

Both areas were located on similiar site conditions, on light clay sands. The PPFP in Luboń emitted the following gases to the atmosphere: oxides of sulphur, calculated as SO_2 – about 40 kg h^{-1} , fluorides, calculated as F – about 11 kg h^{-1} (after Oleksyn and Białobok, 1986). The concetration of fluorides in the air near the provenance trial varied between 0,012 and 0,088 mg F m^{-1} 30 min (Mejnartowicz and Lukasiak,1985). In the 1991 from January to March pollutants

TABLE 2. The main genetic parameters in the particular populations from unpolluted (Kórnik) and polluted (Luboń) area: A/L – mean number of alleles per locus, G/L – mean number of genotypes per locus, He, Ho – heterozygosity expected and observed respectively, F – fixation index, Pg – genotypic polimorphism index. Denotations of the populations as in Table 1.

Populations	A/L	G/L	He	Ho	F	Pg
2. **Russia**						
Kórnik	2,333	2,889	0,3226	0,3148	0,0241	0,4531
Luboń	2,333	3,111	0,3553	0,3519	0,0096	0,4743
3. **Russia**						
Kórnik	2,333	3,000	0,3193	0,3556	-0,1135	0,4309
Luboń	2,444	3,222	0,3464	0,3519	-0,0158	0,4568
4. **Latvia**						
Kórnik	2,444	3,222	0,3430	0,3222	0,0606	0,4766
Luboń	2,444	3,111	0,3180	0,3667	-0,1531	0,4299
5. **Poland**						
Kórnik	2,444	3,111	0,3346	0,3704	-0,1070	0,4565
Luboń	2,667	3,444	0,3646	0,3370	0,0755	0,4956
6. **Poland**						
Kórnik	2,444	3,222	0,2918	0,2704	0,0736	0,4242
Luboń	2,333	3,111	0,3284	0,3296	-0,0038	0,4388
7. **Poland**						
Kórnik	2,333	3,222	0,3325	0,2815	0,1534	0,4644
Luboń	2,667	3,444	0,3515	0,3370	0,0411	0,4941
8. **Poland**						
Kórnik	2,444	3,222	0,3353	0,3716	-0,1084	0,4542
Luboń	2, 667	3,667	0,3861	0,3889	-0,0072	0,5220
12. **Germany**						
Kórnik	2,333	3,222	0,3317	0,2852	0,1401	0,4578
Luboń	2,556	3,333	0,3354	0,3593	-0,0710	0,4570
14. **France**						
Kórnik	2,222	3,000	0,3536	0,3778	-0,0684	0,4627
Luboń	2,444	3,333	0,3605	0,3222	0,1061	0,4914
15. **Sweden**						
Kórnik	2,667	3,444	0,3284	0,3148	0,0412	0,4541
Luboń	2,444	3,000	0,3432	0,3630	-0,0575	0,4783
16. **Slovakia**						
Kórnik	2,444	3,111	0,3506	0,3296	0,0599	0,4847
Luboń	2,556	3,333	0,3528	0,3741	-0,0604	0,4783
17. **Hungary**						
Kórnik	2,333	3,111	0,3009	0,2815	0,0645	0,4205
Luboń	2,333	2,889	0,3371	0,3175	0,0582	0,4545
18. **Jugoslavia**						
Kórnik	2,333	3,000	0,3395	0,3704	-0,0908	0,4612
Luboń	2,333	3,111	0,3356	0,2741	0,1833	0,4726

TABLE 3. The main parameters of studied populations (mean values and ranges): A/L – mean number of alleles per locus, G/L – mean number of genotypes per locus, He, Ho – heterozygosity expected and observed respectively, F – fixation index, Pg – genotypic polymorphism index.

Localization	A/L	G/L	He	Ho	F	Pg
Kórnik	2,39 2,22-2,67	3,14 2,89-3,44	0,33 0,29-0,35	0,33 0,27-0,38	0,13 -0,11-0,15	0,45 0,42-0,48
Luboń	2,48 2,33-2,67	3,24 2,89-3,67	0,35 0,32-0,39	0,34 0,27-0,39	0,10 -0,15-0,18	0,47 0,43-0,52

were emitted in the following amounts: fluorides – 774,5 kg, sulphur oxides – 672 kg, phosphate dusts – 666,9 kg (after Pilc, 1991). Dust and gases emitted by the PPFP affected also the content of phosphorous in the soil (Maszner, 1979). The quantities of this substances were several times greater in comparison with similiar unpolluted soil.

On the basis of electrophoresis of isoenzymes from winter buds (collected in winter 1993/94), an analysis of allele and genotype frequency in the following enzyme systems was made: FEst, ShDH, ADH, GDH, 6PGD, MDH, GOT. The separation of isoenzymes on starch gels and the genetic interpretation of the results were performed as described by Muona and Szmidt (1985) and Prus-Głowacki (1986). In order to determine the genetic structure of the two groups of trees from Kórnik and Luboń , the following genetic parameters were calculated: frequency of alleles and genotypes, observed and expected heterozygosity (H_o and H_e), fixation index (F), genotypic polymorphism index (P_g), as well as genetic similarities and distances according to Nei (DN) and Hedrick (DH), which were used for the construction of dendrites and dendrograms (El-Kassaby, 1991; Hedrick 1974; Jain and Workmann, 1967; Nei, 1975; Nei and Roychoudry, 1974).

RESULTS

The variation of nine isoenzymatic loci (FEst, ShDH A and B, ADH, GDH, 6PGD, MDH C, GOT A and B) in a group of 770 individuals of *Pinus sylvestris* coming from a provenance trial in Kórnik and Luboń was analysed (Tab. 1).

The genetic parameters have (in the most cases) higher values in the populations exposed to heavy pollution (Tab. 2 and 3). In those populations a marked increase in the mean number of alleles per locus (in 7 out of 13 analysed populations), and in the mean number of genotypes per locus (in 9 out of 13 populations) was observed.

The genetic structure, expressed by observed heterozygosity (H_o), fixation index (F) and genotypic polymorphism (Pg), shows that some populations exposed to pollution are characterized by a greater genetic diversity in relation to the analogical populations grownig on the unpolluted area (Tab. 2). In nine populations higher values of observed heterozygosity (H_o) were noted (populations 2, 4, 6, 7, 8, 12, 15, 16, 17). The highest values of this parameter in Luboń were observed in populations from Poland-Rychtal (0.39), Slovakia (0.37) and Latvia (0.37).

10 out of 13 analysed populations from the polluted area are characterized by a higher genotypic polymorphism (P_g) than the control populations. The highest values of Pg were observed in Polish populations (Rychtal – 0.52; Miłomłyn – 0.50; Spała – 0,49).

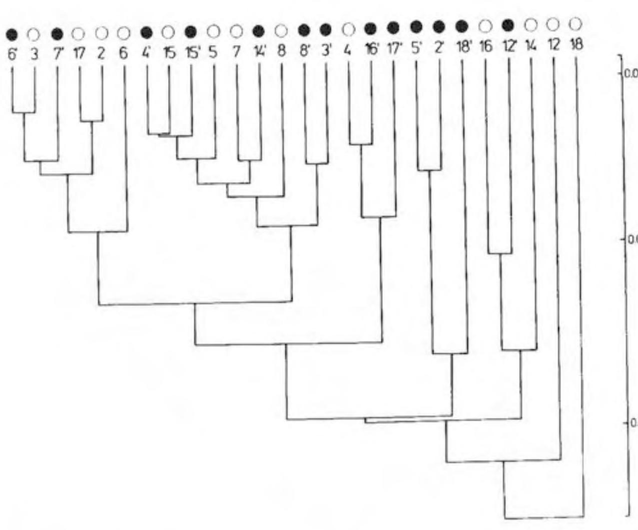

Fig. 2. Genetic similarities between populations based on allele frequency according to Nei:
❍ 2-18 – populations coming from the area free from pollution (Kórnik),
● 2-18 – populations coming from the polluted area (Luboń)

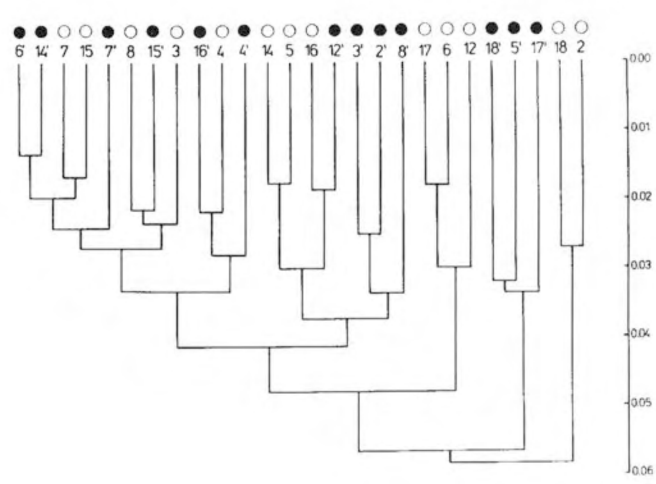

Fig 3. Genetic similarities between populations based on genotype frequency according to Hedrick:
❍ 2-18 – populations coming from the area free from pollution (Kórnik),
● 2-18 – populations coming from the polluted area (Luboń).

The groups of populations from Kórnik and Luboń deviate from Hardy-Weinberg equilibrium because of a considerable excess of homozygotes (Tab. 2 and 3). However, populations from the polluted area are closer to Hardy-Weinberg equilibrium (F = 0,13 and F = 0,1 respectively).

Dendrograms which were constructed on the basis of allele frequency according to Nei and genotype frequency according to Hedrick, did not show genetic identity between the analogous populations at Kórnik and Luboń. This sugests that the genetic structure was changed most probably by pollution factor (Fig. 2 and 3).

DISCUSSION AND CONCLUSIONS

Industrial air pollution has influence upon the gene pool of populations of *Pinus sylvestris* by elimination of some genotypes.

Populations growing in a polluted area are characterized by a higher mean number of alleles and genotypes per locus (A/L and G/L), higher heterozygosity (Ho), and a higher genotypic polymorphism index (P_g). The highest number of alleles and genotypes per locus is found in populations from Poland (Miłomłyn – 4, Spała – 6 and Rychtal – 7), Slovakia – 11 and Germany – 8. In addition, Polish populations have the highest genotypic variation. This means that they are characterized by a great genetic variation, which may be the reason for the fact, that selection coefficient (K), which takes into account of the ratio of tree hight to its survival rate, is more advantageous in Polish than in German, Belgian, Hungarian and Lithuanian populations. The K coefficient is higher in polluted areas (Oleksyn et al. 1988). These authors reported that Polish populations have relatively high survival rates in the heavily polluted area.

Both groups of populations (from the polluted and control area) have excess of homozygotes in comparison to values expected by Hardy-Weinberg equilibrium, although at Luboń the fixation index shows that the excess of the homozygotic genotypes is lower than in Kórnik (Tab. 2).

The genetic structure of populations growing in the polluted area is being changed, which is reflected especially in the dendrograms showing genetic similarities of the studied trees populations (Figs. 2 and 3).

The data presented by Oleksyn et al. (1994) point that ability to maintain a viability population of *Pinus* sylvestris in a polluted environment may be related to genetic richness. The results obtained in this paper suggest that populations which are more genetically variable are more tolerant to industrial pollution. These conclusions correspond to result of Prus-Głowacki and Nowak-Bzowy (1989) obtained for *Pinus sylvestris* populations from regions under strong and weak anthropogenous pressure. They show, that adaptive strategy of the two populations is connected with an increased level of genetic variability (Ho and Pg), relatively higher in the polluted population.. Similiar results were obtained by Mejnartowicz (1983) in *Pinus sylvestris* population and by Prus-Głowacki and Godzik (1995) for *Picea abies* populations, which show higher heterozygosity and genotypic polymorphism in the group of trees tolerant to industrial pollution.

Earlier reports about differential responses to industrial pollution depending on the geographical origin of the population also find confirmation here (Oleksyn et al. 1992a, b). In other work Oleksyn et al. (1986) indicate, that differential sensitivity of *Pinus sylvestris* populations to toxic gases depends on the trees productivity and intensity of photosynthesis.

The data mention above support the classical hypothesis of heterozygotes superiority formulated by Lerner (1954) in a more heterogenous environment plants with a higher degree of genetic multiplicity have a higher viability.

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ZMIANY STRUKTURY GENETYCZNEJ POPULACJI *PINUS SYLVESTRIS* L. NARAŻONYCH NA ZANIECZYSZCZENIA PRZEMYSŁOWE

STRESZCZENIE

Analizie zmienności izoenzymatycznej poddano pąki zimowe *Pinus sylvestris* pochodzące z drzew z doświadczenia proweniencyjnego (IUFRO 1982) w Kórniku i Luboniu założonego z tej samej puli nasion. Populacje w Luboniu poddane były działaniu zanieczyszczeń przemysłowych z zakładów nawozów sztucznych, zaś populacje w Kórniku traktowano jako kontrolne.

Główne parametry określające strukturę genetyczną badanych populacji – częstość alleli na locus (A/L), częstość genotypów na locus (G/L), heterozygotyczność obserwowana (Ho) i spodziewana (He), polimorfizm genotypowy (Pg) – wykazują podwyższone wartości dla populacji poddanych skażeniom. O przebudowie struktury genetycznej tych populacji świadczy również współczynnik Wrighta F (fixation index) wskazujący na tendencję selekcji genotypów homozygotycznych oraz dendrogramy według Nei'a i Hedricka.

Uzyskane wyniki pozwalają stwierdzić, iż populacje charakteryzujące się większą różnorodnością genetyczą wykazują większą tolerancję na skażenia przemysłowe.

SŁOWA KLUCZOWE: *Pinus sylvestris*, proweniencje, struktura genetyczna, tolerancja na zanieczyszczenie powietrza.