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Pollen Viability and Seed Set of Silver Fir (Abies alba Mill.) in Polluted Areas of Slovakia

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Abstract

Viability parameters of pollen grains collected from 16 trees of silver fir (*Abies alba* MILL.) growing in 3 locations in Slovakia under varying degrees of air pollution were compared. In vitro pollen germination tests were compared with controlled crossing experiments. The percentage of germinating pollen grains and pollen tube length varied considerably between individual trees. Both these characteristics were highest in samples from a relatively unpolluted locality (Jedlove Kostolany). Germination of pollen samples from a moderately polluted locality (Repiste) was reduced by 20 %, and in samples from highly polluted habitats (Mociar and Kamenec) germination was reduced by 76 % and 84 %, respectively. In 3 samples of 9 trees from the highly polluted locations, the pollen completely failed to germinate in vitro. Pollen tube length was reduced by 21 %, 15 % and 33 % of the control mean pollen tubes length in samples from the unpolluted site. Except for 2 maternal trees of silver fir which exhibited an inverse relationship, a positive correlation was observed between the in vitro viability parameters of pollen grains and the amount of filled seeds in trees used in artificial crossing experiments.

Key words: Abies alba, pollution, pollen viability, controlled crossing, seed set.

FDC: 181.52; 425.1; 174.7 *Abies alba*; (437).

Introduction

The detrimental effect of air pollution on vegetative parts of plants is a well known and a broadly documented phenomenon. It predominantly refers to changes in leaves at the biochemical, macroscopic and microscopic levels leading to the death of individuals and entire communities (WOLTERS and MARTENS, 1987). In addition, though not so conspicuous, are the effects of air pollution on the reproductive processes in plants. All stages of the reproductive cycle have been shown to be susceptible to air pollutants (SMITH, 1981). At the gametophyte level, it is usually pollen production and viability that are often affected, resulting in a lowered seed set or even a complete absence of seed. Both the direct effect and indirect influence of air pullutants on pollen is believed to be implicated in such cases.

Chronic exposure to high levels of contaminants has resulted in reduction of both pollen viability and number of seeds per cone in *Pinus strobus* and *Pinus resinosa* (HOUSTON and DOCHINGER, 1977) as well as in the reduced size of pollen grains in *Pinus sylvestris* (SHKARLET, 1972; FEDOROV et al., 1983). The indirect effects of pollutants on the microgametophyte are believed to be mediated by changes in the composition of the stigmatic apparatus of female flowers by modifying the biological interaction between pollen and the stigmatic surface (WOLTERS and MARTENS, 1987). Regardless of the way in which the air contaminants affect pollen, the genetic consequence of contamination is a change in competition between the pollen grains on the style resulting in reduction the genetic variation of the next generation (COX, 1984). In spite of this, many antropogenic air pollutants have existed for 100 years and they already serve as a strong natural selection factor in the evolution of the forest ecosystems (SINCLAIR, 1969). It is not surprising that genetic control of the response to air pollution has been postulated (KARNOSKY et al., 1989). The species-specific reaction of pollen grains to acid rain simulated in vitro has been observed by COX (1983). SCHOLZ et al. (1985) have also reported of tree to tree variation in pollen sensitivity of Scots pine to SO_2. MULCAHY (1979) shares the opinion that it is possible to screen the tolerance of sporophyte to the air pollution by selecting the pollen grains that are tolerant to environmental stresses.

As the most endangered species of forest trees in central Europe, the silver fir (*Abies alba* MILL.) has been shown to respond to artificial fumigation with SO_2 by an increased germination of pollen in vitro. Its reproductive organs were injured only when the needles of the same twigs were visibly affected (BEDA, 1982). In spite of these encouraging findings, the fact remains that natural stands of the species suffer in heavily polluted areas not only from dessication, but also of a lack of seed production.

In order to attempt to clarify this situation, a comparative study of pollen viability was performed using the samples of trees from both the heavily and moderately polluted areas as well as from a relatively unaffected locality of silver fir in Slovakia. At the seed level, the biological implications of air pollution on pollen functions was tested by using the same pollen in controlled crossing experiments.

Material and Methods

The study of pollen viability involved 16 individuals of silver fir growing at 4 different habitats in Slovakia which differ in the degree of air pollution and their extent of injury.

The localities of Mociar and Repiste are situated near the aluminium producing factory in Ziar nad Hronom which is one of the largest point-sources of pollution in the country. Mociar is heavily polluted, whereas Repiste is only moderately polluted, according to an emission map of the region. Another locality compared is that of Kamenec pod Vtacnikom located in the vicinity of the industrial center of Novaky with a chemical factory and a coal burning power station. The locality itself is known to be heavily affected by an extremely high level of air pollution where the natural stands of silver fir are restricted to the individual trees or small groups of trees which have survived chronic selection pressure of exposure to pollutants. As a control, the locality in Jedlove Kostolany was used which is considered to be relatively free of air contaminants and whose natural stands of silver fir do not manifest any visible symptoms of injury. The number of trees sampled at the individual test sites, their reference number and characteristics of localities are given in *table 1*.

The 2 separate experiments were performed during the period of 1991 to 1992 with the 4 individuals of silver fir at the locality Repiste and 1 tree in Jedlove Kostolany involved in the 1991 trial, and 11 individuals sampled in Kamenec, Mociar and Jedlove Kostolany in the 1992 experiment.

Strobili were collected from individual trees shortly before shedding of pollen at the end of April and were left at room temperature (20 °C) for 2 to 3 days. This was followed by 1 additional day of drying the pollen which had been sieved and stored over silica gel. After 2 weeks' storage in a refrigerator (4 °C), individual samples of pollen were assayed in 3 separate Petri dishes on a medium consisting of 1 % (w/v) agar and 10 % (w/v) sucrose. On this medium at an incubation temperature of 26 °C it was

Table 1. — Identity of silver fir individuals and localities involved in experiment.

Number of tree	Location	Degree of pollution	Collection date
A. alba 1	J. Kostolany	free of contamination	3 May 1991
A. alba 2	J. Kostolany		6 May 1992
A. alba 3	J. Kostolany		6 May 1992
A. alba 4	Repište	moderately polluted	6 May 1991
A. alba 5	Repište		6 May 1991
A. alba 6	Repište		4 May 1991
A. alba 7	Repište		4 May 1991
A. alba 8	Močiar	densely polluted	6 May 1992
A. alba 9	Močiar		2 May 1992
A. alba 10	Močiar		2 May 1992
A. alba 11	Kamenenc	densely polluted	13 May 1992
A. alba 12	Kamenec		
A. alba 13	Kamenec		
A. alba 14	Kamenec		
A. alba 15	Kamenec		
A. alba 16	Kamenec		

Table 2. — Viability parameters of pollen grains in individual trees of silver fir as revealed by in vitro tests.

Number of tree	Locality	Year of testing	Germination (%)	Pollen tube	
				Mean length (microns)	Coefficient of variation
1	J. Kostolany	1991	84 ± 23	632,10	38,20
4	Repište	1991	83 ± 22	574,50	43,80
5	Repište	1991	66 ± 17	526,50	48,09
6	Repište	1991	45 ± 10	448,40	66,53
7	Repište	1991	81 ± 16	462,26	63,65
2	J. Kostolany	1992	70 ± 18	605,14	31,23
3	J. Kostolany	1992	65 ± 19	676,72	24,84
8	Močiar	1992	15 ± 4	514,26	37,77
9	Močiar	1992	0	0	0
10	Močiar	1992	18 ± 5	578,23	38,82
11	Kamenec	1992	14 ± 3	444,88	27,68
12	Kamenec	1992	10 ± 2	272,24	40,56
13	Kamenec	1992	5 ± 1	517,36	22,86
14	Kamenec	1992	16 ± 3	490,80	24,23
15	Kamenec	1992	0	0	0
16	Kamenec	1992	0	0	0

possible to elongate pollen tubes for 48 hours during which the germination percentage and pollen tubes length were recorded. The proportion of germinating pollen grains was measured from a random sample of 100 pollen grains. The length of pollen tubes in 30 pollen grains was measured in a random sample in fields under the microscope.

The germination tests were compared with the crossing experiments in which 6 individuals of silver fir growing at the localities in Repiste (No. 4, 6, 7) and Mociar (No. 8,9,10) were used as maternal trees. Except for selfing and open pollination, the trees were control cross-pollinated using pollen from trees in the same locality as well as those from the control locality in Jedlove Kostolany. A summary of combinations tested by artificial hybridization is given in *table 3*.

The isolation and artificial pollination as well as extraction of seeds from mature cones was performed according to the procedures described earlier (Kormutak, 1985). The quality of hand-extracted seeds of each cross was determined by X-ray in accord with Czechoslovak standard No. 48 1211 (number of filled seeds in a sample of 400 seeds). The data obtained so far were processed statistically by the t-test and z-test (Smelko and Wolf, 1977).

Results

The viability of pollen grains varied considerably between individuals of silver fir, but in most cases it was positively correlated with the degree of air contamination at the test sites. Of the 2 parameters investigated, pollen germination percentage was found to be more sensitive to the pollution than the growth of pollen tubes. Individuals at the control locality Jedlove Kostolany produced the highest number of germinating pollen grains.

In the 1991 trial, the germination percent of pollen grains originating from the control individual No. 1 reached the level of 84 %, surpassing the corresponding characteristics from individuals No. 4 and 7 at the moderately polluted site (83 % and 81 % germination) but deviating significantly from 2 additional individuals at the same locality (No. 5 and 6) with only 66 % and 45 % of viable pollen produced ($z=2.0^{+}$ and 4.42^{++}; *Table 2*). Germination percent in the control individuals was not reduced, but the pollen tubes achieving on average 632.1 microns contrasted with the reduced rate of pollen tube growth in all the 4 individuals in the moderately polluted site. The data in *table 2* shows that pollen from the control individual (No. 1), has grown statistically significantly more than in 2 of the 4 individuals involved (No. 6 and 7; $t=2.88^{++}$ and 2.11^{++}). No statistical differences of such a magnitude among the individuals at the moderately polluted locality were found.

Similar tendencies were also found in the results of the 1992 study with the exception that the differences between the germination percentage of pollen grains from the control locality and those from the densely polluted stands were much more profound. The viability of pollen grains originating from the 2 individuals in control site reached levels of 70 % and 65 % germination as compared with only a negligible germination in samples from the stands at heavily polluted localities. Accordingly, viable pollen grains derived from the six trees of these localities ranged within the limits of 5 % to 18 % deviating significantly from the percentage of germination established within the pollen samples No. 2 and 3 from the control site ($z=7.69^{++}$ to 10.92^{++}). A complete loss of pollen germinability was found in individuals No. 9, 15 and 16 what may be taken as evidence of a much more drastic consequence of extremely strong pollution exposure at these habitats. Among the differences observed in 1992, only the germination potential of pollen from trees growing under heavy pollution were statistically significant ($z=2.0^{+}$ to 6.16^{++}) except for the pair of individuals No. 11 and 14 which were characterised by similar coefficients of germination of their pollen (14 % and 16 %).

The highest pollen germination percentage in control individuals No. 2 and No. 3 was accompanied by the longest pollen tubes lengths corresponding to 605.14 and 676.72 microns, respectively. The elongation of pollen tubes in samples from the heavily polluted sites was in most cases significantly retarded ($t=2.15^{+}$ to 11.01^{++}) with the exception of individuals No. 8, 10 and 13 approaching in their pollen tube lengths the values mentioned above ($t=0.5$ to 1.92). The extent of pollen tube elongation retardation observed at the heavily polluted site varied considerably in the mean length of pollen tubes in individual trees (*Table 2*).

At the seed level, the use of samples of pollen in artificial pollination experiments resulted in progeny whose quality varied. A lack of correlation was observed in some cases between the proportions of sound seeds in progenies of individual crosses and the in vitro viability of the pollen used. This is true of the crosses between the maternal trees No. 6 and No. 7 and the paternal trees No. 1 and No. 4 which were characterized by a vigorous in vitro growth of pollen tubes (No. 1) but which produced progeny with a lower number of sound seeds than pollen with a lower viability (No. 4; *Table 3*). Statistically non-significant differences in the mean length of pollen tubes of both the above samples ($t=0.85$) negatively affected the proportions of fully developed seeds in the corresponding crossings of maternal trees No. 6 and No. 7 ($z=4.21^{++}$ and 5.20^{++}). The maternal tree No. 9 produced filled seeds even after self-pollination with pollen which failed completely to germinate in vitro. A positive correlation between the parameters of both the pollen grains and mature seeds had been observed when the differences in pollen viability

Table 3. — The results of artificial crossing of selected individuals of silver fir at localities in Repište and Močiar.

Combinations of trees	Date of pollination	Number		Filled seeds	
		Pollinated female strobili	Collected mature cones	Number per 400 analyzed seeds	Percent
A. alba 4 – selfing	11.5.1991	30	18	168	42,0
A. alba 4 x A. alba 1		52	43	190	47,5
A. alba 4 x A. alba 7		48	36	172	43,0
A. alba 4 – open pollinated		–	27	272	68,0
A. alba 6 – selfing	11.5.1991	6	6	82	20,5
A. alba 6 x A. alba 1		22	18	200	50,0
A. alba 6 x A. alba 4		20	14	270	67,5
A. alba 6 – open pollinated		–	20	229	57,2
A. alba 7 – selfing	11.5.1991	12	11	116	29,0
A. alba 7 x A. alba 1		22	18	127	31,7
A. alba 7 x A. alba 4		10	8	184	46,0
A. alba 7 – open pollinated		–	23	185	46,2
A. alba 8 – selfing	8.5.1992	6	6	164	41,0
A. alba 8 x A. alba 10		13	10	160	40,0
A. alba 8 x A. alba 2		14	12	184	46,0
A. alba 8 – open pollinated		–	4	151	37,7
A. alba 9 – selfing	8.5.1992	26	18	86	21,5
A. alba 9 x A. alba 10		18	16	122	30,5
A. alba 9 x A. alba 2		39	31	220	55,0
A. alba 9 – open pollinated		–	15	108	27,0
A. alba 10 – selfing	8.5.1992	12	11	90	22,5
A. alba 10 x A. alba 8		12	11	152	38,0
A. alba 10 x A. alba 2		12	9	250	62,5
A. alba 10 – open pollinated		–	7	120	30,0

characteristics of the paternal trees reached the level of statistical significance. Among the crossings listed in *table 3*, the combinations of individual No. 4 with the trees No. 1 and 7, where the pollination of female strobili with the pollen grains possessing longer tubes (No. 1), resulted in higher proportions of fully developed seeds. The 47.5 % of filled seeds produced in this cross was not, however, statistically significant relative to the 43 % yield of seeds of the same category obtained in the combination of No. 4 x No. 7. The combinations of maternal tree No. 8 with the pollen trees No. 2 and No. 10 may serve as an additional example of such a relationship. Only the combination of controlled crosses involving maternal trees No. 9 and No. 10 were exceptions in demonstrating the differences in viability parameters of pollen samples No. 2, No. 8 and No. 10 and the corresponding yields of filled seeds (*Table 3*).

This variation in the results of controlled crossing was accompanied by a variation in the quality of seed progenies from open pollination. The amount of filled seed ranged between 27 % and 68 %. The relative proportion of sound seeds varied not only with different maternal trees, but also in relation to other crosses with a given tree. Of the 6 maternal trees tested, pollination surpassed its efficiency in both variants of controlled cross-pollination only in individual No. 4 and 1 of the combinations of controlled cross-pollinations in 2 other individuals (No. 6 and 7), were less effective than controlled cross-pollinations in three individuals (No. 8, 9 and 10).

A common feature of these controlled crossing experiments was the reduced amount of sound seeds from self-pollination of all the maternal trees. This may be attributed to inbreeding depression. The amount of sound seeds in selfed progenies ranged between 20.5% (No. 6) and 42 % (No. 4) as compared with the 31.7 % (No. 7) and 67.5 % (No. 6) from controlled cross-pollinations. The degree of inbreeding depression varied individually, and was less remarkable in the controlled cross-pollination involving individuals No. 4 and No. 8 ($z=0.35$ to 1.75) deviating significantly in this respect in individuals No. 6, No. 7, No. 9 and No. 10 ($z=4.91^{++}$ to 13.46^{++}).

Discussion

In spite of an increasing number of reports referring to the detrimental effects of air pollution on plant reproduction under field conditions, it remains unclear which stages in reproductive process are responsible for the observed reductions in fertility (Barret and Bush, 1991). It is likely that changes in the reproductive behaviour are complex, involving a series of events during gametogenesis and subsequent reproductive development which results in a reduction of pollen viability and seed set (Houston and Dochinger, 1977; Murdy, 1979; Cox, 1988a). Both these aspects were observed in this study.

Although pollen of conifers is less sensitive to changes in pH than pollen of broad-leaved species (Cox, 1988b), the data presented here suggests that both pollen germination and germ tube growth of silver fir are sensitive indicators of atmospheric pollution. Of these 2 measurements of pollen viability, pollen germination correlated more closely with the degree of air contamination. Reduction in pollen germinability was observed in samples which originated from densely polluted localities (Mociar and Kamenec). In spite of the considerable variability of this parameter, ranging from a complete inhibition of the pollen germination to 18 % germination, the trees of these regions were very sensitive to pollution. The corresponding response of trees in the area with a moderate degree of pollution (Repiste) was neither profound nor uniform. Two of the 4 trees tested at this locality approached the pollen germination percentage of the control plants from the control site.

The decrease in germination potential of pollen grains from heavily contaminated localities appeared to be due to a slower growth of pollen tubes, but surprisingly, this was not associated with shorter pollen tube lengths. Samples of higher germinability have often been characterized by a lower value of their pollen tube lengths than the samples of pollen with a smaller proportion of germinating pollen grains. A high frequency of meiotic irregularities has been observed in trees of silver fir in contamined area (Kormutak, 1988). This may be regarded as one of the factors responsible for reduction of germinating activity of mature pollen, but the complete loss of pollen germination is undoubtedly a consequence of a more profound effect of air contamination on microstrobili interferring with the vital processes in the developing microsporocytes. The predispositions of individual trees to pollution seem to play an important role in this respect as well.

It is important to mention that effects of air pollution on reproductive processes, including pollen germination and seed development, belong among the stresses that can be most directly related to the selection processes (McLaughlin and Norby, 1991). This often leads to the loss of biological and genetic diversity in populations and communities of plants (Scholz, 1991; Winner et al., 1991). The inverse relationship between germination potential of pollen and seed set of silver fir observed in 2 individuals in controlled crossing experiments may be of a similar nature. The same phenomenon in white pine was attributed to a better adaptation of its own pollen to sites with lower acidity which despite of their higher viability undergo a negative selection in acid droplet of an ovule. According to Cox (1983) such a gametic selection may serve to limit gene flow from populations not adapted to local conditions contributing to the ecotypic differentiation between populations.

The trees of silver fir in these studies do not seem to represent a typical pattern of fertilization events as evidenced by a positive correlation between the viability of pollen grains and quality of viable seed produced in the remaining four individuals of the species used in controled crossings as female parents. The relationship between both these characteristics illustrates the adverse influence of air pollutants on viability of pollen grains in silver fir resulting in lowered fertilizing abilities and subsequent reduction in seed set. The variable degree of correlative association between the pollen germinability and quality of seeds reflects not only the characteristics of the genotypes involved, but probably also the intricate nature of interaction of pollen grains with the nucellar tissue of the ovules. However, the question of possible effects of contamination at the level of female strobili and the extent of their contribution to the reduction of seed sets remains to be examined.

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Performance of Pinus patula Genotypes Selected in South Africa and Growing in Their Native Mexican Environment

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Abstract

Two tests of *Pinus patula* Schiede and Deppe half-sib progenies from South African seed orchards and superior tree selections were planted in Veracruz, Mexico. Measurements made during the first 3 years after planting included height, diameter, crown with, total number of whorls, branch angle, stem straightness, phenology of leader growth and frost damage.

South African families differed in all traits from the unimproved local controls except branch angle. They were taller, had larger diameters and initiated growth earlier. The precocious growth, however, made them more susceptible to frost damage.

Development differed greatly at the two test sites. At one site family variances were nonsignificant, thus improvement was possible only through selection of the South African population over the control. In contrast, selection of the best individuals of the best South African families would result in substantial gains at the other site.

Phenotypic correlations at the two sites were similar and followed a regular pattern. Age-age genetic correlations (determined only at one site), however, were erratic. With non significant family variances and erratic genetic correlations, the results of early selection are unpredictable.

Key words: Pinus patula, progeny test, heritability, selection response, genetic and phenotypic correlations.

FDC: 232.11; 165.3; 165.4; 174.7 *Pinus patula;* (68); (72).

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Introduction

Pinus patula Schiede and Deppe, is one of the most promising plantation species in Mexico, with a natural range from 18^0 latitude N in the state of Oaxaca to 24^0 N in Tamaulipas. It grows in dense, pure stands or in mixtures with *Pinus pseudostrobus* Lindl., *Pinus teocote* Schiede and Deppe, *Pinus ayacahuite* Ehrenb. and *Abies religiosa* (H.B.K), Cham. and Schl. at altitudes from 2000 m to 3000 m. The discontinous distribution forms a narrow belt on the eastern slope of Sierra Madre Oriental, in the mountains in central Mexico (Eje Neovolcanico) and in Oaxaca. In these regions climate is characterized by average annual temperatures from 10^0 C to 16^0 C with 1000 mm to 2000 mm y^{-1} precipitation (Madrigal, 1967; Wormald, 1975; Vela, 1980). Light frost occurs at higher elevations.