DEVELOPMENT AND VIABILITY OF SILVER FIR POLLEN IN AIR-POLLUTED AND NON-POLLUTED HABITATS IN SLOVAKIA

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ABSTRACT

Microsporogenesis and pollen viability of silver fir (*Abies alba* MILL.) trees growing in a polluted and a non-polluted habitat in Slovakia were compared from squash slides of developing microstrobili and from *in vitro* viability tests of pollen grains. The highest frequencies of meiotic irregularities at both habitats were found in pollen mother cells (PMCs) and tetrads. Trees in the polluted locality had a higher frequency of plasmolysed PMCs than did trees at the control site. The slightly higher level of viability of mature pollen at the control site than at the polluted one was not statistically significant. Rather than showing pollen viability differences between polluted and non-polluted areas, the results indicated that differences between individual trees were typical in both localities, suggesting a genotype-specific response of silver fir trees to air contamination. The relatively high viability of silver fir pollen in a polluted locality was ascribed to the reduction of industrial pollutants from a nearby factory during the previous year.

Key words: *Abies alba* MILL., air pollution, bioindication, meiotic disturbances, microsporogenesis, pollen viability

INTRODUCTION

In addition to the generally recognized foliar browning symptoms of forest trees exposed to air pollution, susceptible trees also have been shown to have a reduced reproductive capacity (KARNOSKY & SCHOLZ 1991). A growing body of evidence indicates that pollen viability is the reproductive function most affected by air pollution (SHKARLET 1972; KARNOSKY & STAIRS 1974; OSTROLÚCKA *et al.* 1995). However, in a study of *Abies* species BEDA (1982) reported increased germination of *Abies alba* pollen *in vitro* in response to artificial fumigation of the twigs with SO_2. According to the author, its reproductive organs were injured only when the needles of the same twigs were visibly affected. Conversely, TRETJAKOVA & BAZHINA (1994) reported a conspicuous decline or even total loss of germination potential of pollen in *Abies sibirica.* This adverse effect was observed not only in visibly affected trees of the species in a polluted area of southern Siberia but also in those individuals which did not show any morphological symptoms of injury. With this background of contradictory findings, we made a comparative study of microsporogenesis and quality of mature pollen in silver fir growing in polluted and non-contaminated localities in Slovakia.

MATERIALS AND METHODS

The cytological study of microsporogenesis and subsequent *in vitro* test of pollen viability involved 7 individuals of silver fir (*Abies alba* MILL.) growing at two different habitats in Slovakia which differ in the degree of air contamination. The locality of Močiar is situated near the aluminum producing factory in Ziar and Hronom that has been for decades considered to be the largest point-source of pollution in the country but which has reduced the amount of emitted pollutants considerably during the last year. As a control, the locality in Jedlové Kostolany was used which is considered to be relatively free of air contaminants. The former locality was represented in the experiment by the four individuals and the latter by three trees.

Samples of developing strobili were collected at 1-week intervals throughout the period of February-March and at 3–4 day intervals during April, 1995. Squash preparations of microstrobili were prepared in 1 % acetoorceine and examined microscopically in 45 % acetic acid. A total of 400 cells of each sample were included during evaluation of microsporogenesis at each collection date.

Viability of mature pollen of individual study trees was assayed by *in vitro* testing on a medium consisting

Table 1 The frequency of meiotic disturbances in individual trees of silver fir growing at two different localities

Tree number	Meiotic stage		
	PMCs	Tetrads	Distorted pollen
	%		
Locality Močiar			
1	20.3	24.0	1.8
2	22.2	33.3	5.0
3	17.6	28.8	2.6
4	16.0	22.2	6.3
Mean	19.0	27.0	3.9
Locality Jedľové Kostolany			
1	10.3	27.2	3.3
2	14.6	19.3	0.7
3	19.0	38.0	4.8
Mean	14.6	28.1	2.9

of 1 % (w/v) agar and 10 % (w/v) sucrose. The samples were incubated at 26 °C for 48 hours, after which the germination percentage and pollen tube length were recorded. The proportion of germinating pollen grains was measured from a random sample of 100 pollen grains; pollen tube length was measured in a random sample of 31 pollen grains. Each sample was present in a triplicate. The data obtained from these measurements were processed statistically by *F*-test and *t*-test.

RESULTS

The comparative study of microsporogenesis in silver fir did not reveal any significant differences in the course of meiosis in either group of study trees. The highest frequency of disturbances was usually observed at the levels of pollen mother cells (PMCs) and tetrads. Table 1 shows that along with the normal PMCs there were also many plasmolysed PMCs with a shriveled cytoplasmic content that had not entered meiosis (see also Figure 1). It was at this stage that the study trees in polluted locality Močiar deviated conspicuously from those at the control site in J. Kostolany. The 19 % average proportion of plasmolysed PMCs at the polluted site was stastistically significant ($t = 2.1^{*}$) relative to the 14.6 % share of shriveled PMCs of the control. Also, at stage metaphase I, a small proportion of cells were found in trees from the polluted locality Močiar containing chromosomes that did not follow the typical pattern but which remained loosely scattered in the cytoplasm instead of being arranged at the equatorial region (Figure 3). The frequency of this deviation was, however, nearly negligible. At the stages corresponding to anaphase I (Figure 4–5), diads (Figure 6) and anaphase II (Figure 7) no atypical behaviour of chromosomes was observed at either locality. Abortion of a relatively high fraction of tetrads occurred in all study trees regardless of the normal course of meiosis (Figure 8). The frequency of meiotic disturbances in tetrads ranged from 19.3 to 38 %, averaging 27 % at polluted locality Močiar and 28 % at the control site (Table 1). Thus both the above localities were very similar with regard to this aspect of meiotic behaviour ($t = 0.08$).

The microspores released from abortive tetrads were of two types, (1) pollen grains of typical size and shape with fully developed air sacs and (2) those with distorted shapes which often lacked air sacs (Figure 9). As a result, the mature pollen produced by individual trees also contained a varying fraction of pollen grains with

Table 2 The percentage of *in vitro* germination of pollen grains in seven individuals of silver fir growing at two different localities

Tree No.	Locality	
	Močiar	Jedľové Kostolany
	%	
1	40 ± 5.5	65 ± 3.3
2	28 ± 1.1	40 ± 8.8
3	32 ± 0.6	31 ± 0.5
4	53 ± 3.2	
Average	38 ± 2.5	45 ± 4.2

Table 3 **The average length of pollen tubes (in microns) revealed in 7 individuals of silver fir at two different localities**

Tree No.	Locality					
	Močiar			Jedľové Kostolany		
	n	$\bar{x}$	*v*	*n*	$\bar{x}$	*v*
1	31	112.5 ± 75.2	0.66	23	225.8 ± 113.0	0.63
2	31	137.6 ± 110.6	0.80	31	171.6 ± 56.8	0.33
3	31	136.5 ± 79.2	0.58	31	117.3 ± 49.8	0.42
4	31	229.7 ± 77.6	0.33			
Average		154.1 ± 85.6	0.59		171.4 ± 83.2	0.46

distorted shape (Figure 10). Their frequency averaged slightly higher in trees growing at polluted locality Močiar ($\bar{x}$ = 3,9 %) than at the control locality.

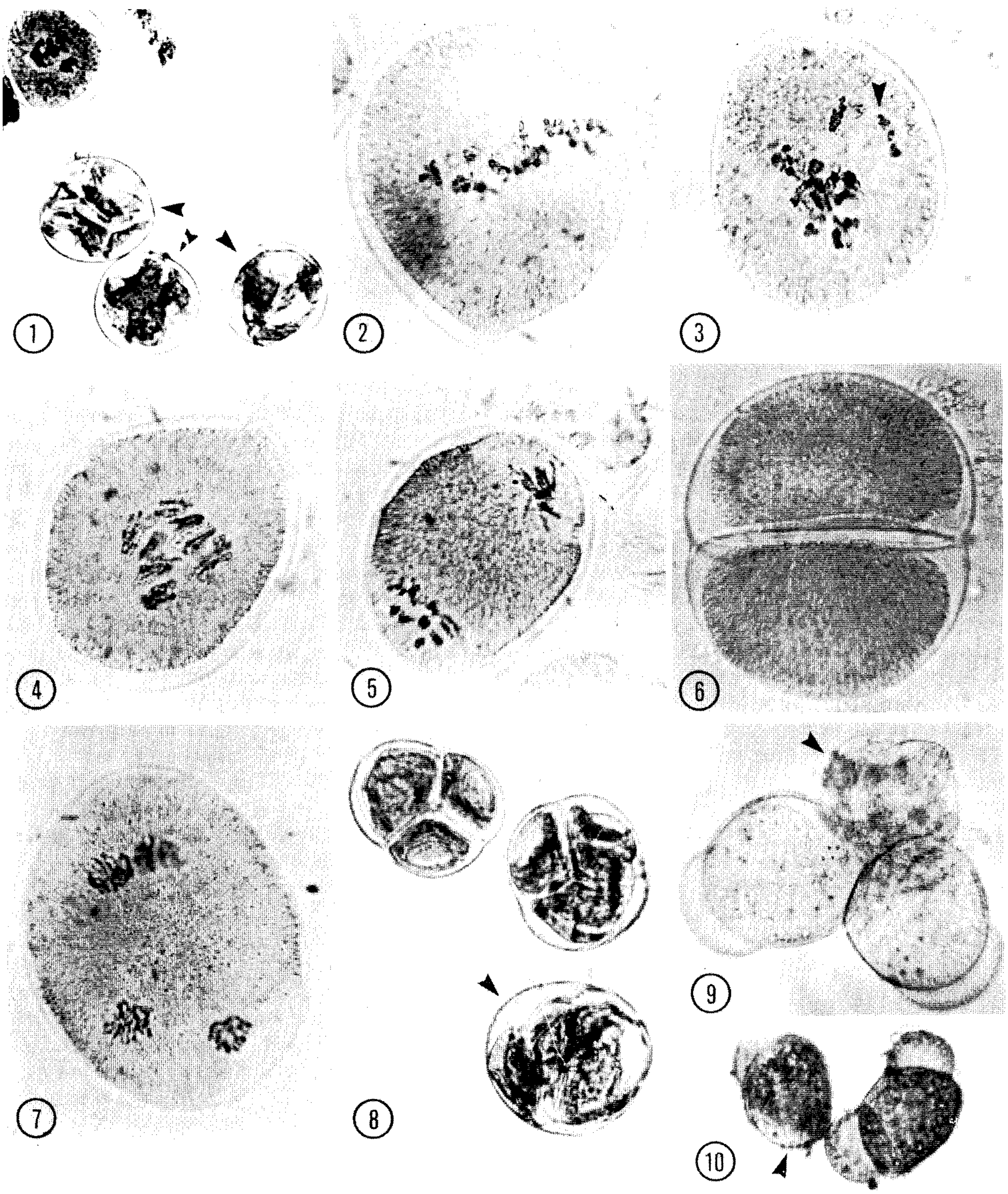
In vitro pollen germination differed slightly between the tree samples from the two localities, averaging 38 % in samples from trees at the polluted locality and 45 % in those from the control area (Table 2). However, in statistical terms this difference between locality means was not significant (t = 0.93). Only the differences between individual trees of a given locality and those between individual trees of both localities compared were significant (t = 0.82–4.35**). Tree 2 of the polluted locality had pollen with the lowest germination (28 %), while Tree 1 from the control site had the highest pollen germination(65 %). Overall, F-tests showed that germination differences among trees were significant (F = 7.73**) whereas the difference between the two localities was not (F = 0.35), although there was a tendency for pollen tubes in samples from the unpolluted locality to grow faster than those from the polluted locality (Table 3).

DISCUSSION

The direct effect of pollutants on pollen, ovules, zygotes and seeds is considered to be the most serious impact of air contamination on plants, resulting in alteration of gene flow and selection and in a change of genetic structure of plant populations (GREGORIUS 1989, GIANNINI & MAGNANI 1994). Of the reproductive structures mentioned, pollen has been reported to be sensitive to air pollutants in concentrations lower than that required for foliar injury (COX 1987). Pollen from trees affected by air pollution has a decreased viability and germination capacity (BELLANI *et al.* 1988). This generalization applies to the pollen of silver firs growing at Močiar, where germination was found to be drastically reduced in 1992 when the pollution of the region was very dense (KORMUŤÁK *et al.* 1994). There was a total lack of pollen germination in one of the study trees and only 15 % and 18 % germination in samples of two additional trees, as compared with 28–54 % in trees of the same locality in 1995, after the emission level from the nearby factory was reduced substantially. This may be taken as one line of evidence of the direct effect of air pollution on viability of silver fir pollen under conditions of natural contamination.

Although statistically non-significant, the persisting differences over time in the viability of pollen from the control locality in J. Kostolany and from the polluted site in Močiar suggest a relatively high sensitivity of silver fir pollen to air contaminants. Of the two characteristics of pollen viability tested so far, germination seems to reflect the quality of pollen better than does pollen tube elongation. It may be noted that TRETJAKOVA & BAZHINA (1994) recommended that pollen tube growth of *Abies sibirica* be used as a biological indicator of air contamination.

Contrary to the small differences observed between the two localities concerned, the variation between individual study trees was large at all the levels studied. Except for the PMCs, which were more affected at the polluted locality, the percentages of abortive tetrads and distorted pollen grains varied in individual trees irrespective of their origin. The same type of variation and comparable frequencies of meiotic irregularities were observed by FEDORKOV (1995) in Scots pine under contamination, and by HUGHES & COX (1993) in two species of birch. TRETJAKOVA & BAZHINA (1994) found a variable degree of pollen viability in affected trees of *Abies sibirica.* Studies have shown a wide variation in the level of reduced pollen viability in a species, ranging from complete inhibition of pollen germination in some trees to 83 % in other trees in the same locality. The same was true of the growth of pollen tubes in individual study trees. According to SCHOLZ *et al.* (1985), the profound intraspecific variability in susceptibility of forest trees to air contamination suggests that in addition to the better-known viability selection, fertility selection occurs in forest stands as well. It is



Figures 1 – 10 Meiotic irregularities of silver fir in an air-polluted environment: **1** – plasmolysed (arrows) and normal PMCs, **2** – typical metaphase I, **3** – atypical metaphase I with chromosomes loosely situated in the cytoplasm (arrow), **4, 5** – anaphase I, **6** – diad, **7** – anaphase II, **8** – typical and abortive (arrow) tetrads, **9** – typical and distorted (arrow) pollen grains after release from tetrads, **10** – pollen grain with typical shape and with missing air sac (arrow).

believed that this type of variability may be effectively utilized in breeding of forest trees for a higher degree of tolerance to air pollution.

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