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Pollen size and viability in hybrid swarm populations of *Pinus mugo* Turra and *Pinus sylvestris* L.

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Abstract: Pollen size and viability in two hybrid swarm populations of *Pinus mugo* and *P. sylvestris* were evaluated using microscopic approach and in vitro germination test. Pollen size of the hybrid swarms was shown to be reduced in comparison with the pollen size of the adjoining control populations of *P. mugo* and *P. sylvestris*. The differences in pollen germination percentage of hybrid swarms at Habovka and Suchá Hora and control population of *P. mugo* at Roháče have not reached the level of statistical significancy. Statistically significant were only differences in pollen tube length. Hybrid swarms exhibited reduced pollen tubes of 15 % and 31 % in comparison with control populations. This indicate reduced vitality of pollen in hybrid swarms. The conclusion has been drawn postulating partial rather than complete sterility of the hybrid swarms.

Keywords: *Pinus mugo*, *P. sylvestris*, hybrid swarms, pollen, size, viability.

Introduction

The two subgenera of the genus *Pinus* seem to be genetically differentiated not only with respect to species crossability but also with regard to fertility of their interspecific hybrids. The soft pines (subgenus *Haploxylon* and/or *Strobus*) are very prone to hybridize mutually with embryo inviability acting as the principal reproductive barrier between species (KRIEBEL 1972). On the contrary, the hard pines (subgenus *Diploxylon* and/or *Pinus*) are much more differentiated genetically involving gametophytic incompatibility as a powerful mechanism of reproductive isolation between species (BUCHHOLZ 1944, MCWILLIAM 1959). The consequence of a higher hybridological afinity among soft pines is a higher degree of fertility of their interspecific hybrids than in the hard pines. SAX (1960) has reported of 11 % of pollen sterility in *P. griffithii* × *P. strobus* and of 30 % of sterile pollen in *P. parviflora* × *P. strobus* hybrids of the soft pines as compared with the 40-50 % share of sterile pollen in the hard pine hybrids of *P. contorta* × *P. banksiana*. We have also found considerable reduction of pollen viability in the two hybrid swarm populations of *P. mugo* and *P. sylvestris* in northern Slovakia reaching the level of 40-41 % of sterile pollen (KORMUŤÁK et al. 2007).

In continuation with these experiments the study on pollen viability has been undertaken involving both pollen size and pollen germinability parameters in some other populations of the hybrid swarms and of *P. mugo* species in the region. The objective of the study was to validate the conclusions about reduced pollen viability in the hybrid swarms on broader experimental and geographical bases. This aspect of *P. mugo* and *P. sylvestris* hybrid swarm populations has until now been neglected, the attention being preferentially oriented towards morphometric and anatomic traits of the needles (BUSINSKY 1998, BORATYŃSKA et al. 2003).

Materials and methods

Material

The study on pollen viability has included the hybrid swarm populations of Scots pine (*Pinus sylvestris* L.) and Swiss pine (*P. mugo* Turra) at Habovka and Suchá Hora in the western part of the High Tatras. The population of *P. mugo* at Roháče adjoining the hybrid swarm at Habovka was used as a control. The location of individual populations is given in Tab. 1. The pollen samples of 30 trees of *P. mugo* at Roháče, 15 trees of putative hybrids at Habovka and 27 trees of putative hybrids at Suchá Hora were collected in spring 2007. Mature microstrobili of individual trees were harvested shortly before shedding of pollen and then transferred to the laboratory. Pollen was extracted from desiccated microstrobili by sieving. The dry pollen was stored in a desiccator over silica gel at 4℃ for the period of 3 weeks and than used in germination test.

Tab. 1. Species and populations used in the experiment

Species/Hybrids	Locality	Altitude	Latitude	Longitude	Collect. year
P. sylvestris	O.Biely Potok	644 m	49°17'12"	19°33'18"	2006
P. mugo	Štrbské Pleso	1355 m	49°07'10"	20°03'41"	2006
Hybrid swarm	Habovka	815 m	49°16'25"	19°37'14"	2006
P.mugo	Roháče	1562-1720 m	49°12'27"	19°44'30"	2007
Hybrid swarm	Habovka	815 m	49°16'25"	19°37'14"	2007
Hybrid swarm	Suchá Hora	765 m	49°23'20"	19°47'11"	2007

In vitro germination

Pollen germination was tested at 25℃ on medium consisting of 1.5 % agar and 10 % sucrose. Each sample was triplicated. After 48 h the number of germinating pollen was recorded from a sample of 100 pollen grains, whereas the pollen tube length in a sample of 30 pollen grains of each Petri dish.

Pollen size measurement

In study on pollen size variation a 1-year stored pollen was used following its acetolysis in a mixture of acetic acid and sulphuric acid (ERDTMAN 1943). Study involved the pollen samples which originated from 12 trees of each the hybrid swarm population at Habovka, *P.mugo* population at Štrbské Pleso in the central part of the High Tatras and *P. sylvestris* population at Oravský Biely Potok adjoining the hybrid swarm at Habovka (Tab. 1). The measurement of the pollen corpus breadth and height was done microscopically using 50 pollen grains of each tree (Fig. 1).

Statistics

The differences in pollen size and in pollen tube length and germination percentage between localities and trees were tested by nested ANOVA. Because the percentages were bimodally distributed, the germination data were stransformed using the arcsin transformation ($p' = \arcsin \sqrt{p}$). All calculations were done using the GLM procedure of SAS (SAS 1988).

Results

Comparative study on pollen size revealed substantial differences between *P. sylvestris* and *P. mugo* species. *P. mugo* pollen was shown to be larger than pollen grains of *P. sylvestris* (Tab. 2) but the hybrid swarm population at Habovka possesses the smallest pollen grains approaching in this respect *P. sylvestris* rather than *P. mugo.* No differences between *P. sylvestris* and *P. mugo* were found in the pollen corpus breadth/height ratio both species sharing the same ratios. Having little lowered ratio the hybrid swarm at Habovka has however deviated from the parental species in this parameter.

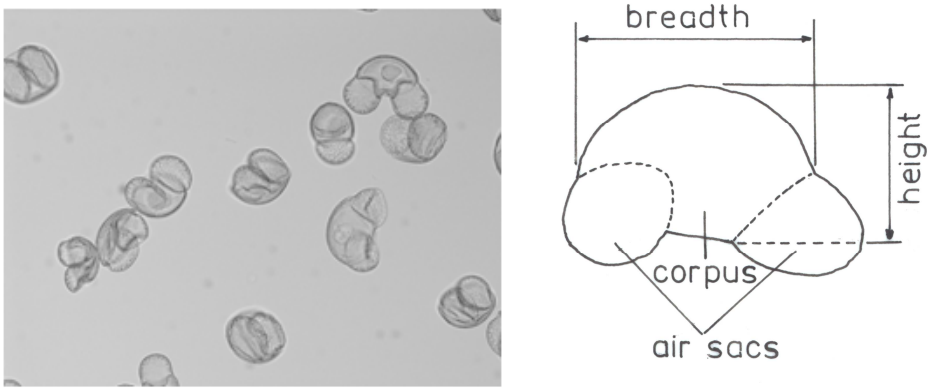

Fig. 1. Acetolysed pollen grains of *P. mugo* (left) and schematic drawing of pollen grain with traits measured (right).

It follows from Tab. 3 that differences between investigated populations were statistically significant with regard to the pollen corpus breadth (F 7.02**). The same was true of the pollen corpus height parameter (F 5.07*). On the contrary, the differences in the pollen corpus breadth/pollen corpus height ratio between individual populations have not reached the level of statistical significancy (F 1.55). It is worth of mentioning in this connection that all the three pollen size parameters investigated differed significantly between individual trees of the respective populations indicating profound individual variation in pollen size (Tab. 3).

Tab. 2. Pollen size in hybrid swarm at Habovka and in populations of parental species

Species/Hybrid	N	Pollen corpus breadth (µm) Mean ± SD	Pollen corpus height (µm) Mean ± SD	Breadth/height ratio
P. sylvestris	600	43.58 ± 3.91	33.75 ± 3.84	1.30 ± 0.14
Hybrid swarm	600	42.42 ± 4.23	33.41 ± 4.02	1.28 ± 0.15
P. mugo	600	45.47 ± 4.83	35.21 ± 4.52	1.30 ± 0.14

Tab. 3. Variance analysis of pollen corpus breadth in hybrid swarm at Habovka and in parental species

Source	D. f.	Sum of squares	Mean square	F value	Variance component [%]
Populations	2	2844.0	375278.0	7.02**	21.0
Individuals	33	6681.3	32499.2	13.12***	33.8
Error	1764	27222.4	400.1		39.9

Pollen viability tests has been preceded by a study on sucrose concentration effect in cultivation media. Among five concentrations tested the 10 % content of sucrose was found to be optimal for in vitro germination of *P. mugo* pollen (Tab. 4).

Tab. 4. Effect of sucrose concentration in cultivation medium on germination of *P. mugo* pollen (The pooled data from two trees)

Sucrose conc. [%]	N	Germination [%] Mean ± SD	N	Pollen tube length [µm] Mean ± SD
3	600	84.16 ± 1.60	180	81.51 ± 23.58
5	600	84.33 ± 2.87	180	87.71 ± 22.25
10	600	92.00 ± 1.09	180	99.51 ± 26.13
13	600	82.16 ± 2.31	180	76.92 ± 27.11
15	600	79.00 ± 9.38	180	71.08 ± 17.84

N – sample size referring to germination percentage (600) and to pollen tube length (180)

As far as pollen viability of hybrid swarm populations at Habovka and Suchá Hora is concerned both populations were shown to produce less viable pollen than control population of *P. mugo* at Roháče. The total amount of 62.24 % and 67.21 % of germinating pollen in hybrid swarms represents reduction of pollen viability of 5 % in the hybrid population at Suchá Hora and of 12 % at Habovka in comparison with the pollen germinability in *P. mugo* (Tab. 5). In spite of these differences the variance analysis confirmed statistical significancy of the differences at the individual level only (Tab. 6). The reduction of pollen germination potential in hybrid swarms has been paralleled by slowered pollen tube growth as evidenced by the average pollen tube length of 67.79 µm in hybrid swarm population at Habovka and of 55.44 µm length at Suchá Hora. The corresponding value in *P. mugo* population averaged at 80.41 µm deviating profoundly from the hybrid swarms (Tab. 7).

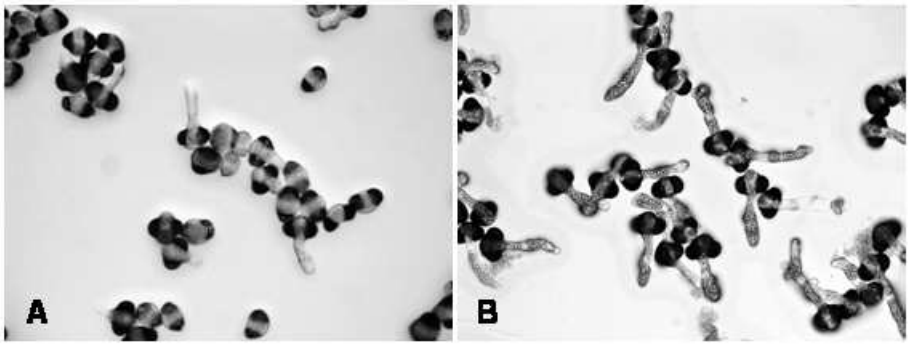

Fig. 2. Germinating pollen of hybrid swarm at Suchá Hora (A) and *P. mugo* at Roháče (B)

Tab. 5. Pollen viability parameters in hybrid swarms and in *P. mugo*

Species/Hybrids	Locality	Germination [%]		Pollen tube length (µm)	
		N	Mean ± SD	N	Mean ± SD
P. mugo	Roháče	88	70.46 ± 17.96	2640	80.41 ± 27.78
Hybrid swarm	Habovka	45	62.24 ± 23.66	1350	67.79 ± 36.93
Hybrid swarm	Suchá Hora	84	67.21 ± 17.41	2520	55.44 ± 19.65

Tab. 6. Variance analysis of pollen germinability in hybrid swarms and in *P. mugo* population

Source	D.f.	Sum of squares	Mean square	F value
Populations	2	0.25999945	0.12999972	1.47
Individuals	13	1.15153096	0.08857930	34.37***
Repetitions	116	0.29898530	0.00257746	0.9534
Error	28	0.11460826	0.00409315	
Corr. total	216	9.87887122		

Tab. 7. Variance analysis of pollen tube length in hybrid swarms and in *P. mugo* population

Source	D.f.	Sum of squares	Mean square	F value	Variance component [%]
Populations	2	750556.0	375278.0	11.55***	21.0
Individuals	70	2274950.5	32499.2	81.22***	33.8
Error	6437	2575673.7	400.1		39.9
Corr. total	6509	5601180.2			

Discussion

Among the aspects related to pollen viability in *P. sylvestris* and *P. mugo*, the studies aiming at optimizing cultivation media composition (CHIRA 1964), long-term storage of pollen (CHIRA 1971, OSTROLUCKÁ et al. 2003) and pollen viability variation at different stands in Slovakia (OSTROLUCKÁ et al. 1995) should be mentioned. Several studies have also appeared referring to pollen abortion and viability variation due to air pollution (FEDOTOV et al. 1983, FEDORKOV 1995, MIČIETA & MURIN 1998). Comparison of our experimental data with those published by other authors revealed some discrepancies which concern both characteristics studied, i. e. pollen size and pollen viability. CHIRA (1971) has for example reported of the non-acetolysed pollen corpus breadth ranging between 62.7 µm and 85.8 µm in *P. sylvestris* and between 62.7 µm and 75.9 µm in *P. mugo.* Taking into account relatively strong genetic control of this characteristic of the pollen, the observed discrepancy may be due to acetolysis treatment leading to destruction of the internal content of the pollen grains and to subsequent shrinking of the pollen corpus size. Obvious seem only the reasons of reduced pollen size in hybrid swarm populations which may be ascribed to the disturbed microsporogenesis in the swarms. It was shown in our previous study that increased frequency of meiotic disturbances occurring in hybrid swarm population at Habovka during anaphases I and II has resulted in a high frequency occurrence of aborted tetrads and aborted pollen grains leading to profound decline of pollen viability in 2005 and 2006 harvests (KORMUŤÁK et al. 2007). The same applies also for the 2007 harvest when the pollen germinability parameters of the hybrid swarms at Habovka and Suchá Hora have not reached the level of those ascertained in control population of *P. mugo* at Roháče. Especially it is true of the pollen tube length characteristics which may be taken as a convincing evidence of the lowered vitality of the hybrid swarms pollen.

Though statistically non-significant but optically conspicuous differences in germination percentage between hybrid swarms and control population of *P. mugo* may be ascribed to the detrimental effect of climatic conditions on microsporogenesis at high elevation of the locality Roháče (Tab. 1). A low amount of functional pollen under high mountain conditions is suggested to be very common (STERN & ROCHE 1974). In case of *P. mugo* population this amount has averaged at 70.46 % which is much lesser than pollen germinability observed at the localities Štrbské Pleso and Popradské Pleso which are located at lower elevations and where pollen germinability averaged during 2005-2006 at 82.83 % and 78.38 % respectively (KORMUŤÁK et al. 2007). It is worth of mentioning that CHIRA (1971) has reported of 60 % germinability of freshly collected pollen of *P. mugo* whose origin has not however been given. Using pollen samples from 7 localities of the High Tatras OSTROLUCKA & FLEISHER (1995) have illustrated both annual and site variations in germinability of *P. mugo* with germination percentage ranging between 48 % and 76 % and pollen tube length averaging at 82-123 µm. Profound effect of climatic conditions on the course of microsporogenesis in forest trees and resulting annual variation in quality of mature pollen seem to be a reasonable explanation of the outlined differences in pollen viability of *P. mugo* at Štrbské Pleso and Popradské Pleso as well. According to degree of sterility STEBBINS (1950) divided interspecific hybrids of plants into two groups. The first group is represented by the hybrids capable of producing some viable pollen and seed set through selfing, intercrossing between F_1 individuals or backcrossing to the parental species. The second group involves the hybrids which are completely sterile. Presented experimental data provided evidence of partial rather than complete sterility of the hybrid swarm population of *P. mugo* and *P. sylvestris*. This may be taken as an additional support for the conclusion of SAYLOR & SMITH (1966) about partially reduced reproductive capacity of the interspecific hybrids of hard pines.

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