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POLLEN VIABILITY IN HYBRID SWARM POPULATIONS OF *PINUS MUGO* TURRA AND *P. SYLVESTRIS* L.

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Pollen viability was tested in two hybrid swarm populations of *Pinus mugo* and *P. sylvestris* in northern Slovakia and in control populations of the parental species. It was significantly reduced in hybrid populations, as evidenced by average germination percentages ranging from 49.0% and 61.53% and by pollen tube length averaging 74.54–86.47 μm. The corresponding values in the control populations were 78.38–88.5% and 102.92–152.84 μm, respectively. The frequency of microsporogenesis disturbances at the tetrad and mature pollen stages was higher in hybrid swarms than in the control population of *P. sylvestris*. Based on in vitro germination data, the amount of sterile pollen was estimated at 40–41% in hybrid swarm populations, 12% in *P. sylvestris*, and 21% in *P. mugo*.

Key words: *Pinus sylvestris* L., *P. mugo* Turra, hybrid populations, pollen viability.

INTRODUCTION

Hybrid sterility is one of the most significant characteristics that discriminates interspecific from intraspecific hybrids. Stebbins (1950) divided sterile interspecific hybrids into two groups: those capable of producing some viable pollen and seed set through selfing, intercrossing between F1 individuals or backcrossing to the parental species; and those completely sterile. The pines seem to belong to the first group, as evidenced by the partially reduced reproductive capacity of the 22 interspecific combinations of *Pinus* studied by Saylor and Smith (1966). Sax (1960) even reported a nearly normal course of meiosis in three interspecific hybrids of Himalayan (*P. griffithii*), Japanese (*P. parviflora*) and American (*P. strobus*) species of white pines, all of which exhibited vigorous growth and relatively high fertility. Summarizing the results of meiotic investigations in conifers, including hybrids, Andersson et al. (1969) concluded that the number of irregularities is on average higher in interspecific hybrids than in the parental species. In light of these findings, it is reasonable to suppose that the figure varies considerably in individual hybrids depending on the genetic relationships of the parental species.

In the presented study we analyzed this aspect of the reproductive behavior of pine hybrids in hybrid swarm populations of *P. sylvestris* and *P. mugo* occurring in northern Slovakia.

MATERIALS AND METHODS

The study included hybrid swarm populations of Scots pine (*Pinus sylvestris* L.) and Swiss mountain pine (*P. mugo* Turra) at Sucha Hora and Habovka in the western part of the High Tatras, along with *P. mugo* populations at Skalnate Pleso and Popradske Pleso in the central part of the same range. *P. sylvestris* was represented by the population in Oravsky Biely Potok adjoining the hybrid swarm population at Habovka. The locations of the populations and the number of trees investigated are given in Table 1. The study spanned two years, with some samples collected in 2005 and with two collections made in 2006. Mature microstrobili of individual trees were harvested shortly before shedding of pollen and then transferred to the laboratory. Pollen were extracted from desiccated microstrobili by sieving. The dry pollen was stored in a desiccator over silica gel at 4°C.

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TABLE 1. Species and populations used in the experiment

Species/Hybrids	Locality	Altitude	Latitude	Longitude	Year collected	No. of trees
Putative hybrids	Sucha Hora	765 m	49°23'20"	19°47'11"	2005	50
Putative hybrids	Habovka	815 m	49°16'25"	19°37'14"	2005	33
Putative hybrids	Habovka	815 m	49°16'25"	19°37'14"	2006	11
Pinus sylvestris	O. B. Potok	644 m	49°17'12"	19°33'18"	2006	12
Pinus mugo	Š. Pleso	1355 m	49°07'10"	20°03'41"	2005	6
Pinus mugo	P. Pleso	1494 m	49°09'18"	20°04'43"	2005	27

TABLE 2. Pollen viability parameters in hybrid swarm populations and in parental populations

Species/Hybrids	Locality	Year collected	Germination [%]		Pollen tube length [µm]	
			N	Mean ± SD	N	Mean ± SD
Putative hybrids	Sucha Hora	2005	150	60.49 ± 22.39	4500	76.75 ± 36.58
Putative hybrids	Habovka	2005	99	61.53 ± 27.66	2832	74.54 ± 33.93
Putative hybrids	Habovka	2006	33	49.15 ± 31.84	990	86.47 ± 38.75
Pinus sylvestris	O. B. Potok	2006	36	88.50 ± 7.20	1080	102.92 ± 41.04
Pinus mugo	Š. Pleso	2005	18	82.83 ± 10.76	540	152.84 ± 50.08
Pinus mugo	P. Pleso	2005	81	78.38 ± 16.01	2430	137.90 ± 44.40

TABLE 3. Variance analysis of pollen germinability in hybrid swarms and in control populations

Source	DF	Sum of squares	Mean square	F value	Variance component [%]
Year	1	0.222	0.222	0.12	0.00
Pop./Year	4	6.938	1.734	8.35 ***	16.77
Indiv./Pop.	118	24.496	0.208	20.20***	58.94
Error	293	3.012	0.010		24.29

IN VITRO GERMINATION TEST

Pollen germination was tested at 25°C 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, and pollen tube length was measured in a sample of 30 pollen grains of each Petri dish. The differences in pollen tube length and germination percentage between years, localities and trees were tested by nested ANOVA. Because the percentages were bimodally distributed, the germination data were transformed using the arcsin transformation ($p' = \arcsin \sqrt{p}$). All calculations were done using the GLM procedure of SAS (SAS 1988). The respective variance components were calculated using the VARCOMP procedure.

MEIOTIC INVESTIGATIONS

A comparative cytological study of microsporogenesis was made using pollen of six trees of the hybrid population from Habovka and seven trees of the *P. sylvestris* population from Oravsky Biely Potok. The study period (May 2–25, 2006) covered all meiotic stages. The sampled microstrobili were processed by squash technique using 1% acetocarmine. The frequency of meiotic irregularities and pollen aberrations was recorded for each collection. The total sample was 500 cells.

RESULTS

Pollen viability parameters varied considerably at both individual and population levels. In samples from 2005, germinability of pollen from the hybrid swarm populations at Sucha Hora and Habovka was much lower than that of the control populations of *P. mugo* at Štrbske Pleso and Popradske Pleso (Tab. 2). The same was true of pollen from the Habovka hybrid swarm population in 2006, whose germination potential was half that of pollen from the control population of *P. sylvestris* from Oravsky

TABLE 4. Variance analysis of pollen tube length in hybrid swarms and in control populations

Source	DF	Sum of squares	Mean square	F value	Variance component [%]
Year	1	429.6	429.6	0.00	0.00
Pop./Year	4	9100998.6	2275249.7	44.98***	37.30
Indiv./Pop.	118	5968123.6	50577.3	8.49***	17.15
Error	12248	1275286.2	1041.6		45.55

TABLE 5. Frequency of microsporogenesis disturbance and pollen viability in tested trees of hybrid swarm population from Habovka (H) and in control population of *P. sylvestris* from Oravsky Biely Potok (B)

Tree no.	Pycnotic nuclei	Asynchron. anaphase I	Asynchron. anaphase II	Aborted tetrads	Aborted pollen	Germination [%]	Pollen tube length [µm]
H1	0.02			0.06	0.12	14.6 ± 0.57	58.9 ± 22.67
H2	0.02	0.005		0.58	0.22	11.0 ± 3.60	73.4 ± 34.17
H3	0.10			0.05	0.12	34.6 ±12.8	67.4 ± 24,50
H4	0.02		0.01	0.36	0.09	13.3 ± 1.52	62.1 ± 23.23
H5	0.01			0.31	0.08	54.3 ± 8.96	79.1 ± 27.10
H6	0.008			0.12	0.13	35.6 ± 5.03	100.6 ± 29.7
B1	0.03			0.07	0.10	93.0 ± 2.64	118.4 ± 51.6
B2	0.01			0.09	0.08	91.3 ± 2.51	84.6 ± 26.9
B3	0.02			0.05	0.08	83.0 ± 5.56	85.0 ± 27.9
B4	0.02			0.15	0.07	81.0 ± 4.00	109.5 ± 39.0
B5	0.03			0.10	0.06	78.0 ±15.71	88.4 ± 44.0
B6	0.04			0.00	0.02	88.6 ± 2.08	105.7 ± 31.4
B7	0.01			0.03	0.06	9.3 ± 3.51	85.7 ± 28.8

Biely Potok. The minimum and maximum values for germination are even more striking: 1% to 99% in the hybrid populations, and 32% to 98% in the paternal populations of *P. mugo* and *P. sylvestris*.

The lower vitality of pollen from the hybrid populations was also reflected in pollen tube growth. As with pollen germination, pollen tube length was significantly reduced in hybrid swarm populations, reaching only half the length recorded from the control population of *P. mugo* in 2005. In 2006, pollen tube length measured from the Habovka hybrid population reached 80% of the corresponding value from *P. sylvestris*.

Variance analysis confirmed the significance of these differences, suggesting high individual variation of germinability and pollen tube length. At the population level, both pollen viability parameters differentiated the hybrid swarm populations from the *P. mugo* and *P. sylvestris* populations (Tabs. 3, 4). On the other hand, no between-year differences in pollen viability parameters were found, indicating that microsporogenesis followed a stable course in 2005 and 2006.

These findings on the hybrid swarms' reproductive behavior are supported by the results of meiotic study. A few meiotic irregularities were observed in dividing pollen mother cells (PMCs) of the hybrid populations, as evidenced by the 0.005 frequency of asynchronous anaphase I in tree H2 and the 0.01 frequency of asynchronous anaphase II in tree H4 (Tab. 5). No such irregularities were detected in PMCs from the *P. sylvestris* trees. The other irregularities in PMCs, tetrads and pollen grains were found in pollen from tested trees of both the hybrid and control populations.

The only deviations observed at the PMC level were the pycnotic nuclei in some cells, which prevented PMCs from entering meiosis (Fig. 1 a,b). The frequency of this disturbance was relatively high, from 0.8% to 10.4% in the Habovka hybrid population and from 0.3% to 4.6% in *P. sylvestris* (Tab. 5). Other types of postmeiotic irregularities were abortive tetrads, generally more frequent in the hybrid swarm populations (6.2–62.0%) than in the control populations (0–10%). The proportion of abortive pollen grains was slightly higher in the

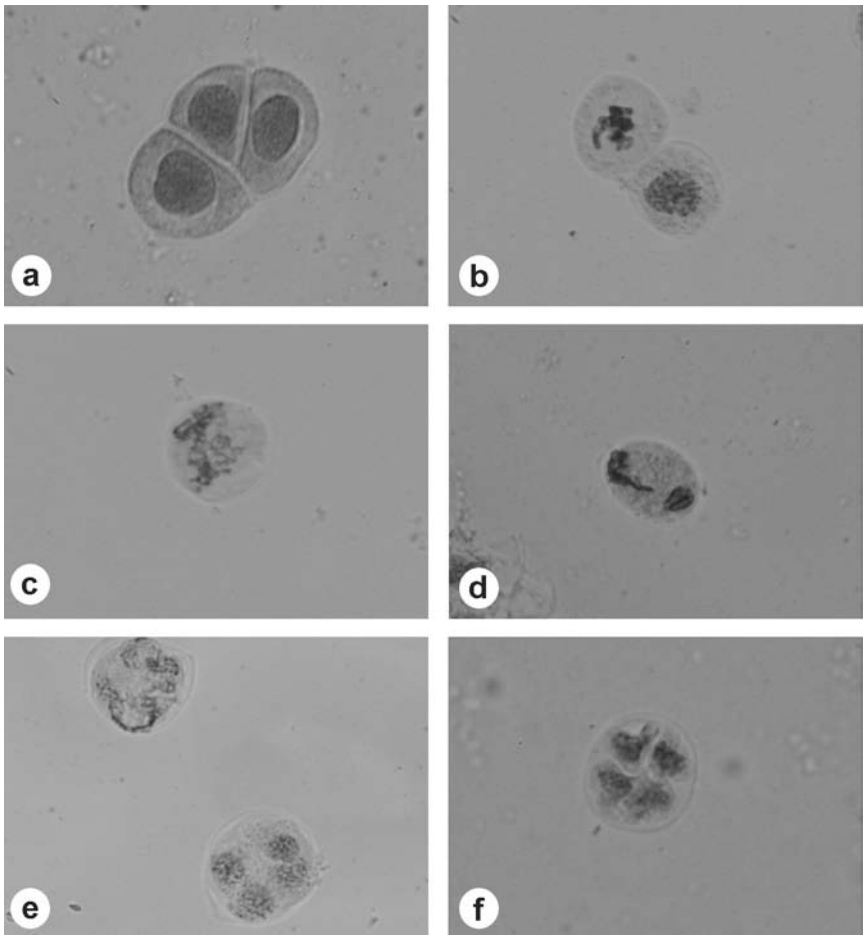

Fig. 1. Disturbances of microsporogenesis observed in pollen from the Habovka hybrid swarm population. (**a**) Normal PMCs, (**b**) PMC with pycnotic nucleus (left) and PMC entering prophase I, (**c**, **d**) Asynchronous anaphase I, (**e**) Asynchronous anaphase II (right) along with microsporocyte with four nuclei, (**f**) Abortive tetrad.

hybrid populations, 8%–22% as compared with 2–10% in *P. sylvestris*.

The observed disturbances of microsporogenesis resulted in significant differences of pollen viability between the hybrids and *P. sylvestris*. Especially remarkable was the statistically significant lower pollen germinability of all the six trees of the hybrid swarm population from Habovka (F 15.63***). Less conspicuous was the reduced pollen tube length of this population as compared to that of *P. sylvestris* (Tab. 5); this difference was not statistically significant (F 3.6).

DISCUSSION

The most recognized places in Europe where hybrid swarm populations of *P. mugo* and *P. sylvestris* have been reported are Rila Planina and Rodopy in Bulgaria (Dobrinov, 1965; Dobrinov and Jahzidis, 1971), the Nowy Targ Valley in Poland (Staszkiewicz and Tyszkiewicz, 1969; Bobowicz et al., 2000), the Swiss Alps (Neet-Sarqueda et al., 1988) and the Orava region in Slovakia (Musil, 1977; Viewegh 1981). Several attempts have been made to demonstrate the hybrid nature of these populations using needle morphology and anatomy (Viewegh and Èambalova, 1993; Staszkiewicz, 1996; Bobowicz et al., 2000), along with needle protein and isoenzyme studies (Prus-Glowacki and Szweykowski, 1980; Prus-Glowacki et al., 1981). However, the fertility of the hybrids has not been studied previously. The present work may be taken as a pilot study in this field, providing direct proof of reduced viability of pollen in spontaneous hybrids of pines. Previous studies on pollen viability in pines aimed to find the appropriate cultivation medium and at optimizing its composition (Chira, 1964). Attention has also been paid to long-term storage of pine pollen, including pollen of *P. sylvestris* and *P. mugo* (Chira, 1971; Ostrolucka et al., 2003). Other studies on *P. sylvestris* pollen estimated its viability at different

sites in Slovakia (Ostrolucka et al., 1995), or examined the process of microsporogenesis and the resulting viability of pollen in trees affected by pollution (Fedotov et al., 1983; Fedorkov, 1995). Our data provide a new look at this aspect of sexual reproduction in pines, focusing on the reproductive potential of introgressive hybrids of *Pinus*. According to Stebbins (1950), interspecific hybrids differ from intraspecific ones by their much more pronounced sterility. In hybrid swarm populations of *P. sylvestris* and *P. mugo* this difference is of a magnitude that places them in the category of partially sterile hybrids. In light of the relatively low occurrence of meiotic irregularities in individuals of the hybrid population, we can only speculate on the nature of the factors causing abortion of such a large number of tetrads and pollen grains. The 58% abortive tetrads detected in pollen from one of the hybrid trees is double the highest number of defects observed in interspecific hybrids of pines by Saylor and Smith (1966). The only difference is that they observed micronuclei in tetrads, whereas plasmolyzed tetrads were the most conspicuous deviation at this stage of microsporogenesis in our study. Except for two hybrids showing 21.8% abortive tetrads, the interspecific hybrids evaluated by Saylor and Smith (1966) shared only 1–2% and exceptionally 3–5% such tetrads. This is much lower than in the hybrid population from Habovka, where the frequency of abortive tetrads was 6–58%. The proportion of aborted pollen in the material from Habovka (8–22%) was comparable to that in the artificial hybrids evaluated by Saylor and Smith (0.45–30.92%; 1966). It is difficult to explain the lack of correlation between the negligible ratio of observed meiotic irregularities and the increased frequency of abortive tetrads and pollen grains and/or reduced viability of mature pollen in the hybrid swarm population from Habovka. According to Sax (1960), the substantial increase in pollen sterility in some pine hybrids, even when meiosis is nearly normal, does suggest that gametic sterility may be caused by incompatible genetic recombinations rather than by irregularities in meiosis. The author pointed out that although meiosis in three F1 hybrids of pines was about as regular as in the parental species, the degree of pollen sterility was higher. In *P. strobus* and *P. parviflora*, pollen sterility was only ?5% as compared with 11% in the hybrid *P. griffithii* × *P. strobus* and 30% in the hybrid *P. parviflora* × *P. strobus*. Our data indicate ~12% sterile pollen in *P. sylvestris* and 21% in *P. mugo*, but as much as 41% sterile pollen in the hybrid population from Habovka and 40% in the one from Sucha Hora. The higher proportion of nonfunctional pollen in *P. mugo* than in *P. sylvestris* may be ascribed to the detrimental effect of climatic conditions on microsporogenesis at high elevations of the High Tatras. A low number of functional pollen under high mountain conditions is suggested to be very common (Stern and Roche, 1974). Both the hybrid swarm populations under study are located below the range of *P. mugo* (Tab. 1), where climatic conditions are not so harsh. Therefore it is reasonable to suggest a genetic background for such a large amount of sterile pollen in these populations. Saylor and Smith (1966) recommend using the frequency of meiotic disturbances in interspecific hybrids of pines as a useful criteria of past introgression of the parental species. We may add that pollen viability is an even better indicator of the introgressive nature of spontaneous hybrids of pines.

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