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In vitro germination dynamics of the common yew (*Taxus baccata* L.) pollen from an autochthonous stand and urban habitats

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Abstract: In 2000 and 2001, *in vitro* pollen viability tests were carried out for common yew (*Taxus baccata* L.) from one point of its natural occurrence (State Nature Reserve Plavno, Slovak Ore Mts., samples marked as PL) and two urban habitats in Zvolen (Central Slovakia) – a park in the downtown (samples marked as ZV) and Borová hora Arboretum (ABH) in the outskirts. The process of the cultivation of pollen can be divided into three essential phases: (1) pollen imbibition and loss of exine; (2) elongation of male gametophyte and (3) development of pollen tube. Concerning the irregular pollen tube formation under *in vitro* conditions, however, both elongated and germinating pollen grains were considered viable. According to this criterion, the mean estimated viability of the pollen from the “worst” locality (center of Zvolen) was half lower compared to the arboretum, regarded as the “best” one. Using the Duncan’s multiple range test, the ascertained differences between the samples from the “best” and the “worst” locality were statistically significant at $\alpha = 0.05$.

Keywords: *Taxus baccata*, male generative organs, pollen, pollution, pollen viability.

Introduction

In addition to changes in the physiological activity of assimilatory organs, the influence of stress on forest woody plants is also reflected in their generative sphere, particularly as a rapid decrease of sexual reproductive ability. One of the most significant indicators of the reproductive ability of plants is, besides the production of vital seeds, the viability of pollen. Regarding the fact that microsporogenesis and male gametophyte formation are considered weak links in the reproductive cycle of coniferous species, many authors have tried to find correlation between the rate of abnormal microspores or decreased pollen viability and real or simulated environmental pollution (a review of older literature was published by WOLTERS & MARTENS 1987, newer authors are e.g. O'CONNOR et al. 1987, COX 1988, KRUG 1990, PAOLETTI & BELLANI 1990, CELA RENZONI et al. 1990, CELA RENZONI & VIEGI 1991, PAOLETTI 1992, KORMUŤÁK et al. 1994, OSTROLUCKÁ et al. 1995, MIČIETA & MURÍN 1998, TRET'YAKOVA & BAZHINA 1994, 2000, TRET'YAKOVA et al. 1996 and others).

In Slovakia, common yew (*Taxus baccata* L.) grows in the lower storey of mixed forest stands in relic-like plant associations referred to as *Taxo-Fagetum* that occur almost exclusively on carbonate bedrock (BLATTNÝ & ŠŤASTNÝ 1959, KORPEĽ & PAULE 1976, PAULE et al. 1993, LUKÁČIK & NIČ 1997, SANIGA 2000 and others). Because of its aesthetic value and relative hardiness to pollution emissions, however, yew is frequently planted in potentially polluted urban habitats. Given its commonly high production of microstrobiles and frequent occurrence in cities, common yew provides good opportunities for studies on the influence of stress conditions on its male generative organs.

Material and methods

Branches with male strobiles were collected from nine male individuals of common yew from three different sites in central Slovakia. One locality contained naturally occurring *Taxus baccata* (State Nature Reserve Plavno in western part of Slovak Ore Mts., central Slovakia – locality marked as PL) while the other two sites were urban habitats with different degrees of air pollution: a park in the center of Zvolen (high pollution, locality marked as ZV) and the Borová hora arboretum on the periphery (lower pollution, locality marked as ABH). The sampling was carried out at the beginning of the vegetation periods in 2000 and 2001, a few days before the start of the pollen shedding.

The outward appearance of male strobiles was investigated by scanning electron microscope (SEM). The samples were fixed overnight with a buffered solution of glutaraldehyde, rinsed with a phosphate buffer and dehydrated by a graded acetone series. After the critical point drying (CPD) procedure, they were coated with a thin layer of gold.

After the shedding of pollen in the lab, a part of the air-dried pollen grains was stored at 4 °C and, after coating with gold without previous fixation, dehydration and CPD, observed directly using SEM. The other pollen samples were grown on 1% (weight/volume) agar medium with the addition of 10% (w/v) sucrose in the dark room. The course of the experiment was evaluated every 24 hours during the following four days. 400 random pollen grains from each individual were assessed under a light microscope.

The numeric data were evaluated using two-way analysis of variance. The arcsine transformation was used because of absence of normal distribution. In addition to the analysis of variance, the Duncan's test was used for the multiple comparisons of mean values.

Results and discussion

Notes to the morphological features of male generative organs

Pollen grains of common yew develop inside the radially arranged microsporangia (Fig. 1a). Five to six microsporangia are distributed around the stalk of the microsporophyll (microsporangiophore), equipped with the 5–6-angular scutellum. 10 to 15 (mostly 13–14) microsporangiophores compose the simple male strobile (Fig. 1b). According to older opinions (FLORIN 1948), the male generative organs of *Taxaceae* are so peculiar in their structure and appearance that, together with the female generative structures with a single terminal ovule instead of a female strobile, this constitutes a basis for splitting off a separate monotypic group from other coniferous plants. According to recent knowledge (MUNDRY & MUNDRY 2001), however, the simple male strobiles of *Taxaceae* should be derived from compound staminate cones, analogical to those of rather similar and probably older *Cephalotaxaceae* (LO & WANG 1999). Because of the conspicuous stalk, the microsporophylls (microsporangiophores) of *Taxus* and related genera should, in this way, represent the strongly reduced simple male strobiles.

The supposed significant relationship between *Taxaceae* and *Cephalotaxaceae* is also supported by genetic analyses that do not confirm a unique position of *Taxaceae* within the system of gymnosperms. On the contrary, their close relatedness to *Taxodiaceae*, *Cupressaceae* and especially to *Cephalotaxaceae* was demonstrated using molecular markers (CHAW et al. 1997, CHENG et al. 2000).

Common yew pollen grains are relatively small and, when dry, have an irregular shape with a diameter of about 16–20 :m (Fig. 1c). They contain only one haploid nucleus, because the prothallial cells are missing and, as it is suggested by the cytological literature data (DUPLER 1917, STERLING 1948, PENNELL & BELL 1986b, ANDERSON & OWENS 2000), primary, secondary and tertiary mitotic division occurs in the male gametophyte as late as during the pollen tube growth.

Course of the cultivation of pollen

After 24 hours of cultivation on agar medium, most of the pollen grains were moderately enlarged, released from exine, and their original irregular shape was changed into the globular one with a diameter of about 18–22 :m (Fig. 1d). Elongation of pollen grains was only sporadic at that time and the pollen tube formation was not observable at all. However, the "swollen" pollen was easily distinguishable from pollen grains with the original irregular shape and more or less visible symptoms of damage (in investigated samples, their rate was usually less than 10%). On the third day of experiment, elongation of the male gametophyte was more frequent with a length to width ratio of approximately 1.5 : 1 (Fig. 1e). In the various points of the elongated male prothallium (but most frequently, on its distal pole), the first pollen tubes started to be observable in the form of small bulges of intine. 72 hours after the start of experiment, the percentage of the elongated pollen grains increased and the mutual ratio of the lengths of their axes was almost 2 : 1 (the mean length of the male gametophyte was approximately 30 :m, whereas its width remained unchanged). The pollen tubes increased in occurrence and length (Fig. 1f). This trend continued during the next 24 hours. After 5 days of cultivation, however, pollen tube formation was usually stopped because of intensive fungal growth.

A similar course of the pollen germination on agar medium was observed in common juniper (*Juniperus communis* L.) from *Cupressaceae* with very similar anatomical and morphological features of pollen grains (KRIŽO & KORÍNEKOVÁ 1985, KORÍNEKOVÁ & KRIŽO 1986).

On the basis of the different behavior on agar medium, the pollen grains were arranged into the following four groups:

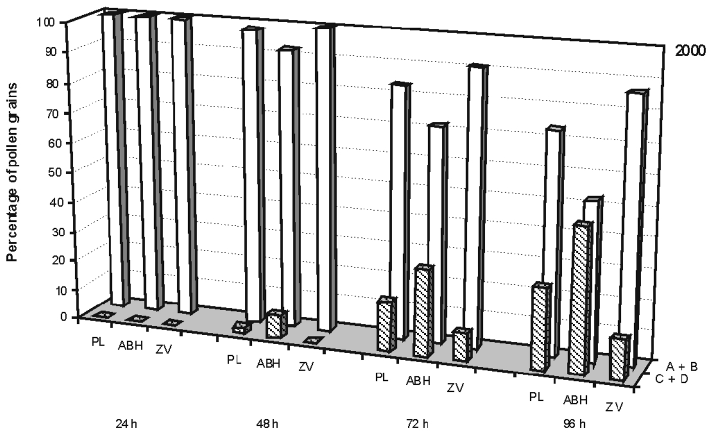
A – pollen grains without any symptom of imbibition of germination, frequently with more or less visible symptoms of damage;

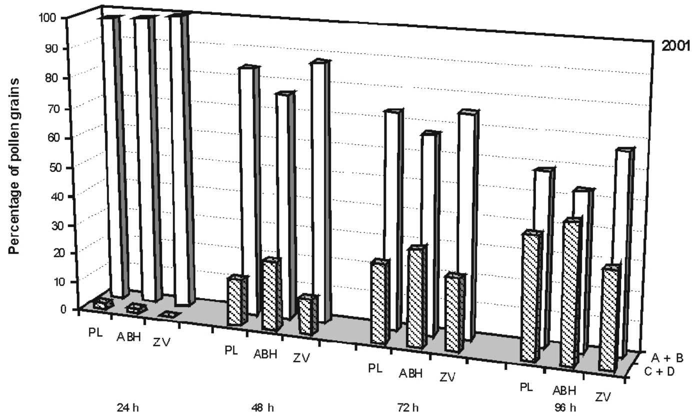
B – globular pollen grains released from exine and increased in size;

C – elongated pollen grains with the minimum axis ratio of 1.5 : 1;

D – tube-producing pollen grains.

Pollen classified as A should be considered unambiguously dead, and the viability of the pollen grains from the B group is doubtful (swelling up of pollen grains and rupturing of exine could be just mechanical processes, caused by an intensive water absorption). On the contrary, the pollen included in D should be characterized as viable. Because of irregular pollen tube formation, however, both elongated and tube-producing pollen grains were regarded as viable. The Figure 2 shows the total germination dynamics of the investigated samples, based on the above-mentioned assumptions.





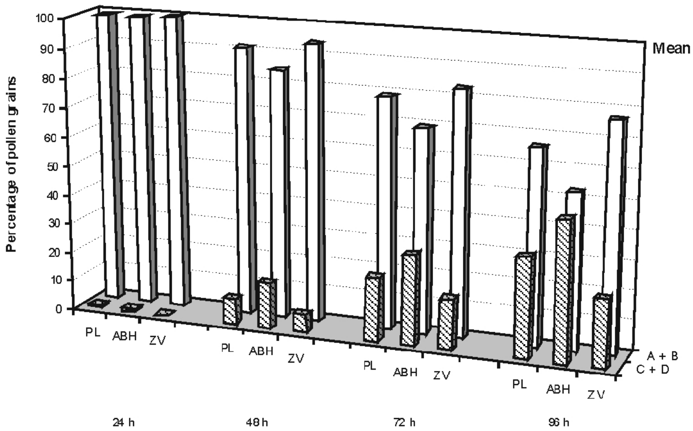


Fig. 2. *In vitro* germination dynamics for the observed pollen samples during individual years (PL – State Nature Reserve Plavno; ABH – Borová hora Arboretum; ZV – park in Zvolen; A + B: pollen grains considered dead or doubtful; C + D: pollen grains scored as being alive).

Comparison of individual samples

The highest mean percentage of the pollen grains from C and D groups (considered viable) was characteristic of individuals growing in the Borová hora arboretum. In the samples from this locality, 47.3% elongated and tube-producing pollen were counted after 96 hours of cultivation on average (47.8% in

2000, 46.7 in 2001). For particular individuals, the values ranged between 26.5% (yew shrub marked as ABH-1 in 2000) and 73.0% (ABH-2 individual in the same year). Conversely, the lowest mean values (13.3% in 2000, 33.0% in 2001, 23.2% in total) were for those samples from the park in the center of Zvolen. Finally, the yew trees from the autochthonous stand in Slovak Ore Mts. with the percentages of viable pollen of 26.7% (2000), 41.3% (2001) and 34.0% (both years of studies) were very close to total mean values for all the localities investigated (29.3% for 2000, 40.3% for 2001, as well as 34.8% in total).

As it is shown in the last column of Table 1, analysis of variance did not demonstrate the existence of significant differences either among the localities or between individual seasons. Nevertheless, the results of Duncan's test (Tab. 2) indicate that after 96 hours of cultivation, the samples from Borová hora arboretum had significantly higher percentage of viable pollen grains than the samples from the center of Zvolen.

Tab. 1. Analysis of variance for transformed rates of pollen grains scored as being viable (C a D group after 96 hours of cultivation).

Source	Degrees of freedom	Sum of	Mean square	*F*	Pr > *F*
Year	1	0.08901675	0.08901675	2.25	0.1597
Locality	2	0.23663080	0.11831540	2.99	0.0885
Year × locality	2	0.05627680	0.02813840	0.71	0.5110
Error	12	0.47525204	0.03960434		
Total	17	0.85717639			

Tab. 2. Transformed means and results of Duncan's test at the significanance level of $\alpha = 0.05$ for rate of the pollen grains scored as being viable (mean values marked with the same letter are not significantly different).

Year	*n*	Mean		Locality	*n*	Mean		
2000	9	0.54315	A	ABH (2000–2001)	6	0.7577	A	
				PL (2000–2001)	6	0.6056	A	B
2001	9	0.68379	A	ZV (2000–2001)	6	0.4771		B

The main cause of the high sensitivity of the male generative structures of woody plants to the influence of environmental stress is probably their long-lasting development. In common yew, the initiation of male strobiles to pollen shedding takes at least 10 months (MUNDRY & MUNDRY 2001). A relatively long duration is also typical of microsporogenesis itself, occurring usually in the late autumn (DUPLER 1917, PENNELL & BELL 1985, 1986a, ANDERSON & OWENS 2000), but sometimes in early spring in urban habitats (KRIŽO & KORÍNEKOVÁ 1989). During the morphogenesis of the male sporogenous tissue and microspores, the male generative sphere is sensitive to stress factor influences because of longer exposition time compared to the developing vegetative organs (FEDOTOV et al. 1983).

Unfortunately, the results of this experiment characterize only the germination dynamics of individual pollen samples over 96 hours and therefore, do not necessarily reflect the real viability of pollen. Regarding this fact, only the relative differences are comparable with literature data.

For the *Pinus pinea* L. pollen samples from a heavily polluted industrial center, CELA RENZONI et al. (1990) detected one-half lower viability compared to individuals from natural sites and also observed the numerous morphological abnormalities. For the Scots pine, FEDOTOV et al. (1983) observed a decrease of pollen viability by one-third as a consequence of increased atmospheric sulphur dioxide concentration. A decrease of one quarter was ascertained for the pollen viability of *Pinus resinosa* AIT. and *Pinus strobus* L. (HOUSTON & DOCHINGER 1977). The percentage of viable pollen was even zero for some individuals of silver fir from heavily polluted industrial localities (KORMUŤÁK et al. 1994). After elimination of the industrial pollution, however, pollen viability increased within a few years and the previous differences between moderately and heavily polluted areas became statistically non-significant (KORMUŤÁK 1996). Pollen viability of *Abies sibirica* LEDEB. differed with altitude, distance from the source of emissions and sulphur accumulation in the assimilatory organs (TRET'YAKOVA & BAZHINA 1994, 2000, TRET'YAKOVA et al. 1996). For *Pinus sylvestris* L., *P. nigra* ARNOLD and *P. mugo* TURRA, frequencies of abortive pollen grains were significantly increased by environmental pollution (MIČIETA & MURÍN 1998).

In simulated environmental pollution studies, pollen viability of conifers was apparently negatively affected by increased acidity (COX 1988, PAOLETTI & BELLANI 1990, CELA RENZONI & VIEGI 1991, PAOLETTI 1992, HOLUB & OSTROLUCKÁ 1993), fumigation of pollen by SO_2 (O'CONNOR et al. 1987 and older authors), as well as the presence of a detergent (PAOLETTI 1992) and some trace metals, especially lead, (COX 1988, HOLUB & OSTROLUCKÁ 1993) in the growth medium.

Several authors (SCHOLZ et al. 1985, TRET'YAKOVA & BAZHINA 1994, 2000, KORMUŤÁK et al. 1994, KORMUŤÁK 1996, TRET'YAKOVA et al. 1996, OSTROLUCKÁ & FLEISCHER 1995, OSTROLUCKÁ et al. 1995, SLOBODNÍK & KRIŽO 1997, FLEISCHER et al. 2000 and others) mention significant differences in total viability of pollen among individuals under the same stress conditions. This fact perhaps testifies to the different sensitivity of genotypes against stress factors and to the possible natural selection of resistant genotypes and the elimination of individuals with decreased male reproductive ability.

Conclusions

After 96 hours of cultivation, the difference between the maximal and minimal percentage of elongated and germinated pollen grains of common yew was 34.5% in 2000 and 13.7% in 2001 (24.1% on average for both years of investigations). Compared to the less polluted Borová hora arboretum, which had the highest percentage of the pollen grains considered viable, the mean values were approximately half as much as samples from the more highly polluted center of Zvolen. Regarding this fact, the viability of the common yew pollen, estimated from the percentage of elongated and germinating pollen grains in individual samples, can be considered a good indicator of the influence of stress factors (above all, pollution of air) on the generative organs. A certain disadvantage, however, concerns the relative difficulty of common yew pollen to produce pollen tubes under *in vitro* conditions. To achieve the more accurate estimation of real pollen viability, a special emphasis should be made on the sterility of samples and maximization of the cultivation time.

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References

Anderson E. D. & Owens, J. N. (2000): Microsporogenesis, pollination, pollen germination and male gametophyte development in *Taxus brevifolia*. – Ann. Bot. 86: 1033-1042.

Blattný T. & Šťastný T. (1959): Prirodzené rozšírenie lesných drevín na Slovensku. – Slovenské vydavateľstvo pôdohospodárskej literatúry, Bratislava.

Cela Renzoni G., Viegi L., Stefani A. & Onnis A. (1990): Different *in vitro* germination responses in *Pinus pinea* pollen from two localities with different levels of pollution. – Ann. Bot. Fenn. 27: 85-90.

Cela Renzoni G. & Viegi L. (1991): *In vitro* sensitivity of *Pinus pinaster* and *P. pinea* pollen grains to different pH values. – Ann. Bot. Fenn. 28: 135-142.

Chaw S. M., Zharkikh A., Sung H. M., Lau T. C. & Li W. H. (1997): Molecular phylogeny of extant gymnosperms and seed plant evolution: Analysis of nuclear 18S rRNA sequences. – Mol. Biol. Evol. 14: 56-68.

Cheng Y., Nicolson R. G., Tripp K. & Chaw S. M. (2000): Phylogeny of *Taxaceae* and *Cephalotaxaceae* genera inferred from chloroplast *mat*K gene and nuclear rDNA ITS region. – Mol. Phylogenet. Evol. 14: 353-365.

Cox R. M. (1988): The sensitivity of pollen from various coniferous and broad-leaved trees to combinations of acidity and trace metals. – New Phytol. 109: 193-201.

Dupler A. W. (1917): The gametophytes of *Taxus canadensis* Marsh. – Bot. Gaz. 64: 115-140.

Fedotov I. S., Karaban' R. T., Tikhomirov F. A. & Sisigina T. I. (1983): Evaluation of SO_2 effects on Scotch pine stands (in Russian). – Lesovedenie (6): 23-27.

Fleischer P., Ostrolucká M. G. & Ludvová A. (2000): Influence of environmental factors on generative reproductive ability of *Picea abies* (L.) Karst. in the Tatra National Park. – Ekol. Bratislava 19: 117-124.

Florin R. (1948): On the morphology and relationships of the *Taxaceae*. – Bot. Gaz. 110: 31-39.

Holub Z. & Ostrolucká M. G. (1993): Tolerance of pollen from trees to chronic effect of Pb and acidity. –Biologia 48: 331-335.

Houston D. B. & Dochinger L. S. (1977): Effects of ambient air pollution on cone, seed, and pollen characteristics in Eastern white and red pines. – Environ. Pollut. 12: 1-5.

Korínekova M. & Križo M. (1986): Príspevok k poznaniu morfogenézy samčích reprodukčných orgánov *Juniperus communis* L. – Acta Fac. For. Zvolen 28: 61-69.

Kormuťák A. (1996): Development and viability of silver fir pollen in air-polluted and non-polluted habitats in Slovakia. – For. Genet. 3: 147-151.

Kormuťák A., Salaj J. & Vooková B. (1994): Pollen viability and seed set of silver fir (*Abies alba* Mill.) in polluted areas of Slovakia. – Silvae Genet. 43: 68-73.

Korpeľ Š & Paule L. (1976): Chránené územie Malé Plavno. – Českoslov. Ochr. Prír. 16: 153-173.

Križo M. & Korínekova M. (1985): The effect of IAA, GA_3 and $GA_{4/7}$ on the microprothallium formation of some conifers. – Acta Univ. Agric. Brno, C 33: 271-275.

Križo M. & Korínekova M. (1989): Mikrosporogenéza tisu obyčajného (*Taxus baccata* L.) v podmienkach stredného Slovenska. – Biologia 44: 21-26.

Krug E. (1990): Reduced fertilization capacity of SO_2-fumigated *Picea omorika* pollen. – Eur. J. Forest Pathol. 20: 122-126.

Lo H. C. & Wang Y. F. (1999): Development of staminate cone and microsporogenesis in *Cephalotaxus wilsoniana* Hay. – Proc. Natl. Sci. Counc. ROC(B) 23: 85-98.

Lukáčik I. & Nič J. (1997): Ekologická charakteristika spoločenstiev prirodzeného výskytu tisu obyčajného (*Taxus baccata* L.), jeho rastové pomery a zdravotný stav. – Acta Fac. For. Zvolen 39: 9-20.

Mičieta K. & Murín G. (1998): Three species of genus *Pinus* suitable as bioindicators of polluted environment. – Water Air Soil Poll. 104: 413-422.

Mundry I. & Mundry M. (2001): Male cones in *Taxaceae* s.l. – an example of Wettstein's pseudanthium concept. – Pl. Biol. 3: 405-416.

O'Connor C. J., Ashford K. P. & Spedding D. J. (1987): Germination and metabolism of *Pinus radiata* pollen in the presence of sulfur dioxide. – J. Pl. Physiol. 126: 373-378.

Ostrolucká M. G. & Fleischer P. (1995): Pollen vitality monitoring in the Tatra Biosphere Reserve. – Ekol. Bratislava 14: 391-397.

Ostrolucká M. G., Bolvanský M. & Tokár, F. (1995): Vitality of pine pollen (*Pinus sylvestris* L.) and *Pinus nigra* Arnold) on sites with different ecological conditions. – Biologia 50: 47-51.

Paoletti E. (1992): Effect of acidity and detergent on *in vitro* pollen germination and tube growth in forest tree species. – Tree Physiol. 10: 357-366.

Paoletti E. & Bellani L. M. (1990): The *in-vitro* response of pollen germination and tube length to different types of acidity. Environ. Pollut. 67: 279-286.

Paule L., Gömöry D. & Longauer R. (1993): Present distribution and ecological conditions of the English yew (*Taxus baccata* L.) in Europe. – In: Yew Resources Conference,.Berkeley, CA, 189-196.

Pennell R. I. & Bell P. R. (1985): Microsporogenesis in *Taxus baccata* L.: The development of the archaesporium. – Ann. Bot. 56: 415-427.

Pennell R. I. & Bell P. R. (1986a): Microsporogenesis in *Taxus baccata* L.: The formation of the tetrad and development of the microspores. – Ann. Bot. 57: 545-555.

Pennell R. I. & Bell P. R. (1986b): The development of the male gametophyte and spermatogenesis in *Taxus baccata* L. – Proc. Roy. Soc. London, Ser. B, Biol. Sci. 228: 85-96.

Saniga M. (2000): Štruktúra, produk┌né a regenera┌né procesy tisu oby┌ajného v Štátnej prírodnej rezervácii Plavno. – J. For. Sci. 46: 76-90.

Scholz F., Vornweg,A. & Stephan B. R. (1985): Wirkungen von Luftverunreinigungen auf die Pollenkeimung von Waldbäumen. – Forstarchiv 56: 121-124.

SLOBODNÍK B. & KRIŽO M. (1997): The pollen germination intensity and the length of pollen tubes of *Picea abies* (L). KARST. from the Protected Landscape Area – Biosphere Reserve Poľ ana (Slovenské Stredohorie Mts.). – Bull. Polish Acad. Sci., Biol. 45: 149-155.

STERLING C. (1948): Gametophyte development in *Taxus cuspidata*. – Bull. Torrey Bot. Club 75: 147-165.

TRET'YAKOVA I. N. & BAZHINA E. V. (1994): Viability of Siberian-fir pollen in disturbed forest ecosystems of the mountains of southern Siberia. – Russ. J. Ecol. 25: 413-420.

TRET'YAKOVA I. N. & BAZHINA E. V. (2000): Structure of crown as well as pollen and seed viability of fir (*Abies sibirica* LEDEB.) in disturbed forest ecosystems of the Khamar-Daban Mts. near Baikal lake. – Ekol. Bratislava 19: 280-294.

TRET'YAKOVA I. N., ZUBAREVA O. N. & BAZHINA E. V. (1996): Influence of environmental pollution by sulfur oxides on the morphological structure of the crown, generative sphere, and pollen viability of the Siberian fir in the Baikal region. – Russ. J. Ecol. 27: 14-20.

WOLTERS J. H. B. & MARTENS M. J. M. (1987): Effects of air pollutants on pollen. – Bot. Rev. 53: 372-414.

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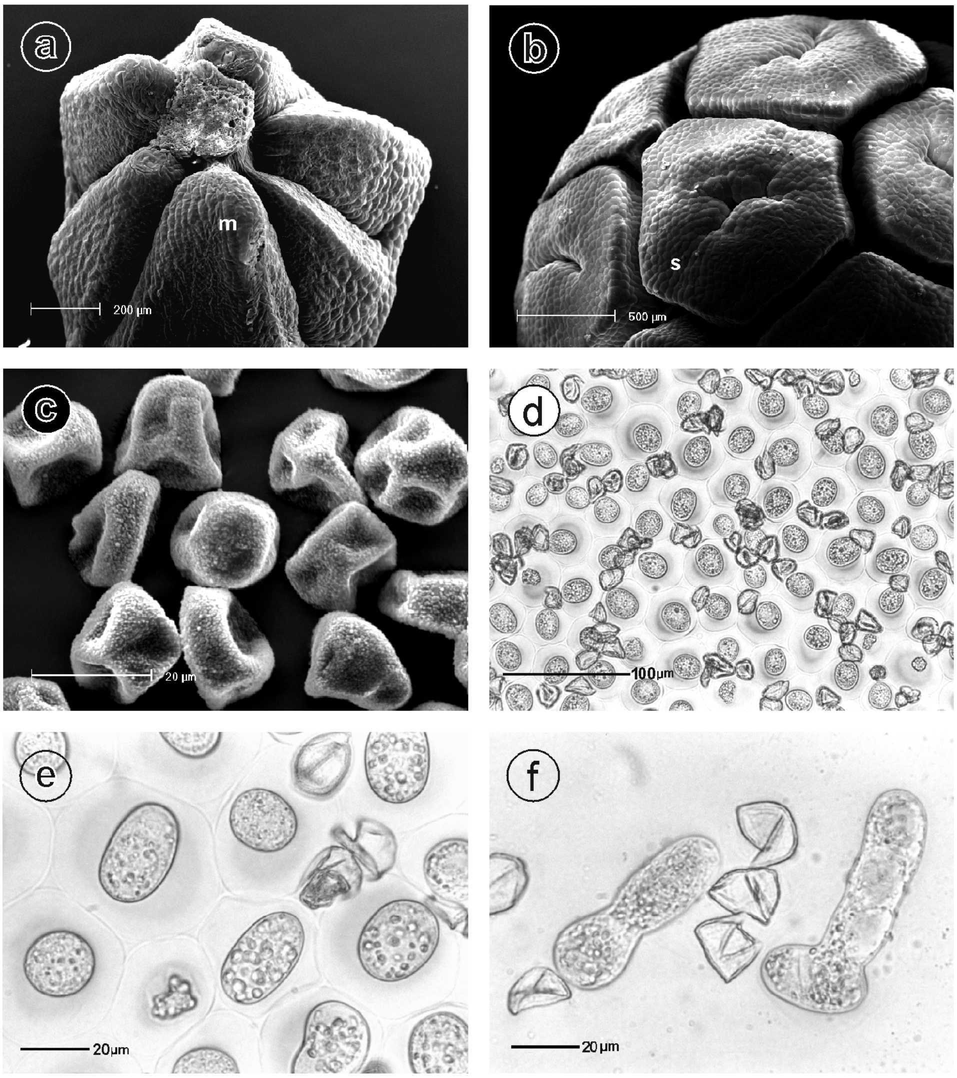

Fig. 1. Morphology of the male generative organs and *in vitro* germination of common yew pollen (m Microsporangium; s Scutellum). a **Microsporangiophore with radially arranged microsporangia (SEM);** b **Microstrobile with microsporangiophores (SEM);** c **Dry pollen grains (SEM);** d **Swollen up pollen grains, released from exine (24 hours of cultivation);** e **Elongated pollen grains (48 hours of cultivation);** f **Pollen grains producing pollen tubes (72 hours of cultivation).**