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Research Article

Morphology and viability of pollen grains of *Picea* L. species in the conditions of introduction

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Abstract

Research was carried out in the Botanical garden of Petrozavodsk state University, located on the Northern shore of the Petrozavodsk Bay of lake Onega (middle taiga subzone, 61°47' n). during two vegetation periods. The objects of study served as an indigenous species *Picea abies* (L.) Karst, and two introduced species – *Picea pungens* Engelm. f. glauca Regel. *Picea canadensis* (Mill.) Britton et al. Age and height of *Picea abies* – respectively 47 years and 16 m, *Picea pungens* – 36 years and 12 m, *Picea canadensis* – 22 years and 6 m. Morphological characteristics of pollen grains were estimated by the following morphometric characteristics: diameter, length of pollen grain, polar axis, height of pollen grain with air bags, area of pollen grain projection, perimeter, form factor and eccentricity. The study of statistical characteristics of samples allowed us to establish that the accuracy rate in determining the arithmetic mean values of the morphometric parameters under study is quite high and varies by species from 0.7 to 3.5%.The pollen grains of the studied species were found to be bag-shaped or wide-ellipsoid. Their outline from the pole is elliptical, from the equator it is wide-trapezoid. The colour of the grains is yellow, the surface of the body is lumpy, and the bags are rough. The pollen grains of the aboriginal *Picea abies* species are 3–20 % larger than those of the introduced species. The size of the *Picea pungens* pollen exceeds that of *Picea canadensis* by 4–48 %. There is a fairly strong linear relationship between individual morphometric parameters of a pollen grain (r = 0.5–0.8). The viability of the pollen of the aboriginal *Picea abies* species in South Karelia is about twice as high as that of the introduced species of *Picea canadensis* and *Picea pungens*.

Keywords

introduction, spruce, pollen, morphology, viability

† *Deceased*

Introduction

The study of the introduced plants resistance to new conditions cannot be limited to observations of external morphological changes occuring in the process of growth and development. It is necessary to clarify the characteristics and internal changes in the vegetative and generative buds, as well as in the strobile. It is on the characteristics of all developmental stages of generative organs and the degree of male and female gametophytes formation that the quantity and quality of seed production depend (Antosiewicz 1961; Nekrasov 1991). Only the selection of specimens grown from the seeds of local introduction and showing the best adaptation to new conditions can ensure successful introduction (Nekrasov 1975; Elagin 1980; Kalutsky et al. 1981). It is required to know the features of the generative sphere development to determine the prospects of an introduced species. One of the most important characteristics of pollen is its viability (Vladimirova et al. 2008; Vorobyev and Teben'kova 2013; Zalyvskaya 2014; Popova et al. 2016). Determining the quality of fruiting plants pollen allows us to assess the degree of their adaptation to botanical gardens conditions and reproductive ability (Tikhov et al. 1990). The extrapollination carried out in botanical gardens or controlled pollination also require a preliminary assessment of pollen viability. A systematic annual analysis of pollen viability usually accompanies traditional phenological observations of the introduced species. When studying this indicator one can easily determine morphological heterogeneity of the subject of research. Large pollen grains are typical of fertile pollen, whereas smaller and shriveled ones are sterile.

Pollen viability and the morphology of pollen grains in Karelia were previously studied for Pinus sylvestris L. (Kozubov 1974; Kozubov et al. 1982), Abies balsamea Mill. (Tikhova and Titova 1987), Larix sibirica (Trenin 1990), Picea abies (L.) Karst. (Kozubov 1974; Kozubov et al. 1982).

The comparative study of pollen grains viability and morphology of two introduced species and a local species of Picea abies was carried out with the aim of determining the reproductive potential of the introduced species.

Materials and methods

The studies were carried out at the Botanical Gardens of Petrozavodsk State University located on the north shore of the Petrozavodsk Bay of Lake Onega (central taiga subzone, 61°47'N) during two vegetative periods. The type of soil is humus-ferruginous-illuvial podzol (pH 4.7).

The subjects of the studies were the aboriginal species of *Picea abies* (L.) Karst and two introduced plants of *Picea pungens* Engelm. f. glauca regel. and *Picea canadensis* (Mill.) Britton et al. The age and height of *P. abies* is 47 years and 16 m, the *P. pungens* is 36 years and 12 m, the *P. canadensis* is 22 years and 6 m.

The viability of pollen grains was determined on fresh and fixed material. Fresh pollen was germinated on distilled water and on 20 % sucrose solution (in three replications). The calculation of fertile and sterile grains and the study of the growth characteristics of pollen tubes were carried out on the 5th day by a light microscope.

The pollen was fixed according to Chamberlain (Furst 1979), stained with acetocarmine and iodine solution (Pausheva 1988). The fresh pollen was poured with Canadian balsam to study the morphological signs. The resulting permanent preparations were studied by a light microscope. The MOV−1-15 eyepiece micrometer was used to measure the parameters of pollen. The electron-microscopic studies were performed in the Laboratory for electron microscopy studies, Department of General Physics of Petrozavodsk State University with the use of the REM-200 scanning microscope. In this case, dry pollen was applied to the object table and sprayed with gold at the VUP-4 instrument.

The measurement results were processed by variation statistics methods (Zaytsev 1984). The significance level is 0.95, the sample size for each indicator is 100.

Results

The vital potential of pollen grains of one or several closely related species can be indirectly estimated by their size: the larger they are, the more vigorous the germination is. The morphological characteristics of pollen grains were assessed according to the following morphometric characteristics: diameter, pollen grain length, polar axis, height of a pollen grain with air bags, pollen grain projection area, perimeter, form factor, and eccentricity (Tikhov et al. 1990). The measurement results are presented in Table. 1.

The pollen grains of the *Picea* species have the same bag-shaped or wide-ellipsoid shape. Their outlines from the pole are elliptical, and from the equator they are wide-trapezoid, sometimes almost triangular (Figure 1). The colour of the grains is dark yellow, the surface of the body is lumpy, and the air sacs are rough.

The variants distribution in samples of all morphological parameters corresponds to the normal distribution law. The study of the statistical characteristics of the samples made it possible to establish that the accuracy rate in determining the arithmetic

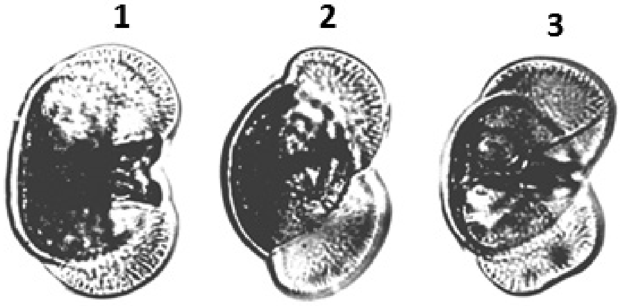

Fig. 1. Microscopy of pollen grains (body and air bags, ×360). *Picea abies* (1), *P. canadensis* (2), *P. pungens* (3)

Table 1. Morphometric signs of pollen grains of various species of *Picea*

Species	Arithmetic mean value error, µm	Measure of the accuracy of experience,%	Standard deviation, µm	Coefficient of variation,%
The Equatorial diameter				
Picea abies	47.2±0.52	2.5	5.22	11.0
P. pungens	45.7±0.40	8.7	3.96	8.8
P. canadensis	44.0±0.29	6.7	2.88	6.4
Length				
P. abies	35.8±0.49	1.4	4.86	13.8
P. pungens	32.6±0.41	1.3	4.14	12.7
P. canadensis	27.9±0.22	0.8	2.16	7.5
The polar axis				
P. abies	30.1±0.40	1.3	3.96	13.3
P. pungens	26.8±0.38	1.4	3.78	13.9
P. canadensis	25.0±0.29	1.2	2.88	11.4
The body height with the air bags				
P. abies	36.9±0.36	1.0	3.60	9.8
P. pungens	33.0±0.36	1.1	3.58	10.8
P. canadensis	29.8±0.62	2.3	2.52	8.1
The area of the projection				
P. abies	1840±60.1	3.5	0.63	18.9
P. pungens	1489±70.3	4.6	0.56	20.8
P. canadensis	1312±10.2	0.8	0.29	13.6
The perimeter of the projection				
P. abies	168±4.3	2.4	0.35	9.6
P. pungens	157±4.3	2.3	0.35	10.6
P. canadensis	106±0.8	7.3	0.81	21.7
Formfactor				
P. abies	16.2±0.01	0.6	0.08	2.0
P. pungens	16.6±0.01	0.6	0.18	2.1
P. canadensis	8.6±0.05	5.9	0.49	12.1
The eccentricity				
P. abies	0.64±0.02	2.5	0.17	20.0
P. pungens	0.59±0.01	1.4	0.13	14.9
P. canadensis	0.57±0.01	1.1	0.12	13.2

mean values of the morphometric parameters under study is quite high and varies by species from 0.7 to 3.5 % (Table 1). It decreases to 5.9–7.3 % only in *P. canadensis* with respect to the perimeter of pollen grain projection and form factor.

The highest variability is noted for such indicators as the area of pollen grain projection and eccentricity. Their variation coefficients vary within 15–21 %, as for the other indicators they are lower and sometimes significantly lower (2–6 %). The trend towards a decrease in variability of linear indicators of pollen grains from *P. abies* to *P. pungens* and *P. canadensis* is clearly expressed.

P. abies was found to have the largest values of the studied signs of a pollen grain. Its equatorial diameter in *P. abies* (47.2 μm) is 3.2 and 7.3 % bigger than in *P. pungens* and *P. canadensis*. According to T.P. Nekrasova (1983) and I.N. Tretyakova (1989, 1990), the value of this indicator in *P. abies* in the conditions of Siberia is close to our results and is respectively 57 and 46 μm.

The body length of pollen grains of *P. abies* is longer than that of *P. pungens* and *P. canadensis* by 10.2 and 28.3 %, the polar axis – by 11.9 and 20.0 %, the height of a pollen grain with air bags – by 11.2 and 23.5 %, the area of pollen grains – by 16.8 and 32.8 %, the value of the perimeter of the projection of pollen grain – by 7.0 and 58.5 % respectively. Thus, the aboriginal species has the largest pollen grain body, which indirectly proves the highest nutrient content contributing to the efficient growth of the pollen tube and fertilization. Significantly larger sizes of pollen grains of the aboriginal species (*P. obovata*) as compared with the introduced species (*P. pungngens*) were previously discovered by E.V. Bazhina and M.I. Sedaeva (2017).

Discussion

The pollen grain form factor (the perimeter squared in relation to the pollen grain area) is statistically the same in *Picea abies* and *Picea pungens* (16.2 and 16.6), but approximately 90 % more than that in *Picea canadensis*.

A comparison of the morphological characteristics of the pollen grain of *P. pungens* and *Picea canadensis* showed that the equatorial diameter of the pollen grain of *P. pungens* is larger than that of *P. canadensis* by 3.9 %, the body length – by 16.8 %, the polar axis – by 7.2 %, the body height – by 11.1 %, the area – by 13.7, and the perimeter – by 48.1 %. The increase in the size of the *P. pungens* pollen grains as compared with *P. canadensis* was also found by E.V. Bazhina and M.I. Sedaeva (2017).

A correlation analysis was performed to clarify the nature and the strength of the relationship between the studied morphometric characteristics of the pollen grain. A linear relationship of different strength was found between some of the studied signs (Table 2). For instance, a rather significant relationship between such indicators of a pollen grain as the polar axis and the height of a pollen grain with air bags (r = 0.7…0.8) was established for all the studied *Picea* species. For *P. abies* a correlation (r = 0.5) was found between the equatorial diameter and body length of a pollen grain, and for *P. canadensis*, in addition, also between the equatorial diameter and the polar axis of a pollen grain.

The studies showed that the pollen tubes of the plants grown on sucrose-bearing medium appear as early as on the 1st day, and on water – only on the 2nd or even the 3rd day. During pollen germination starch grains occupy most of its volume. At the same time,

Table 2. Correlation coefficient between morphometric signs pollen grains of various species of *Picea**

Morphometric feature	Plant species								
	Picea abies			*P. pungens*			*P. canadensis*		
	A	B	D	A	B	D	A	B	D
E	0.54	0.22	0.22	–	0.53	0.52	–	0.30	0.31
A	–	0.23	0.24	0.30	0.22	0.20	0.31	0.0	0.0
B	–	–	0.72	–	–	0.81	–	–	0.71

Note: E – equatorial diameter, A – body length, B – polar axis, D – body height with air bags.

there are far more of them on sucrose than on water. Pollen tubes on sucrose are 2–3 times longer than those germinated on water.

The incubation medium also has a significant effect on pollen viability. This indicator for the aboriginal species germinated on sucrose-bearing medium is 21 % higher than those germinated on water, whereas for the introduced species – by 30–33 %. Similar results were previously obtained by I.A. Smirnov (1977). The viability of *P. abies* pollen is on average twice as high as that of the introduced species. The differences in this indicator between the latter do not exceed 8 %. The pollen viability of *P. abies* germinated on water was found to be 80 %, 97 % – on sucrose-bearing medium; of *P. pungens* – 40–45 %, of *P. canadensis* – 50–60 %. It should be noted that in the homeland of the introduced species (North America) this indicator reaches 90 %. The low viability of these species pollen appears to be related to the adverse ecological conditions of the introduction area (Skutko 1973; Sedaeva et al. 2008; Bazhina 2015). These include late spring and early autumn frosts often observed in Karelia, as well as severe winter frosts. Moreover, the low fertility of podzolic soils together with increased acidity is likely to depress the development of male gametophytes in the introduced species.

Conclusions

1. The pollen grains of the studied species were found to be bag-shaped or wide-ellipsoid. Their outline from the pole is elliptical, from the equator it is wide trapezoid. The colour of the grains is yellow, the surface of the body is lumpy, and the bags are rough.
2. The pollen grains of the aboriginal species of *Picea abies* are 3–20 % larger than those of the introduced species.
3. The size of the *Picea pungens* pollen exceeds that of *Picea canadensis* by 4–48 %.
4. There is a fairly strong linear relationship between individual morphometric parameters of a pollen grain (r = 0.5–0.8).
5. The viability of the pollen of the aboriginal species of *Picea abies* in South Karelia is about twice as high as that of the introduced species of *Picea canadensis* and *Picea pungens*

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