Pollen development and morphology in different Picea A. Dietr. species at the V.N. Sukachev Institute of Forest Arboretum

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ABSTRACT

Pollen development and morphology of four Picea A. Dietr. species at the V.N. Sukachev Institute of Forest Arboretum have been studied. The researches have shown that pattern of pollen development in all four Picea species is largely the same as in other coniferous species. Pollen irregularities were found in 0, 20-27,8 % pollen grains. Ontogenesis of the spruce trees in a non-native climatic environment, such as at the Arboretum showed capability for adjustments to rapidly changing climatic conditions.

Keywords: Picea obovata, P. pungens, P. glehnii and P. mariana, ex situ conservation, pollen development, pollen morphology, irregularities.

Investigations on the adaptation of reproductive processes in plants to changing environmental conditions are extremely important. In this respect, botanical gardens and arboreta provide valuable information on any species and climatypes during conservation ex situ. As known tree species have ability to form climatic ecotypes (climatypes) in the course of its adaptation to local conditions: air temperature, relative humidity, and day length (Pravdin 1964, Nekrasov 1971, Mamaev and Andreev 1996, Yakovlev et al. 2012). These factors can depress reproductive capacity due to severe reduction in the production of fertile, filled seeds (Woodward 1990). The reproductive potential decreases when environmental conditions became non-optimal for climatypes, which is often the case in plant introduction.

High sensitivity to new conditions is characteristic of the beginning of the reproductive phase during sporogenesis and gametogenesis adaptation to new temperature and light regimes (Shkutko 1991, Gavrilon and Butorina 2005). It was established that even slight changes of air temperature can have marked effects on growth and ontogenesis, especially on pollen development (Barner and Christiansen 1960, 1962, Ekberg and Eriksson 1967, Ekberg et al. 1968, Eriksson 1968, Sarvas 1968, Kozubov 1974, Jonsson 1974, Luomajoki 1977, Andersson 1980, Bazhina et al. 2009). The changes of air temperature appeared to be responsible for phenology shift and physiological processes including pollen and seed formation (Rowe 1964, Nekrasov 1971, Kohmann and Johnsen 1994, Strois 2000, Messaoud et al. 2007, Levanic et al. 2009, Bazhina 2014a). The study of Siberian fir (Abies sibirica) at introduction showed that climatic changes induced irregularities of meiosis and pollen development (Bazhina et al. 2011, Bazhina, 2014b). Poor pollination of fir trees was resulted in empty seed forming (Bazhina 2015). Seedling of P. pungens, P. glehnii and P. mariana were grown in 1966 from seeds obtained by seed exchange from native populations. The P. obovata f. seminskiensis seedlings were transferred to the Arboretum in 1972 from the forest nursery of M.A. Lisavenko Research Institute of Horticulture of the V.N. Sukachev Institute of Forest, Central Siberia, Russia.

MATERIALS AND METHODS

The study was conducted in 2008 in the Arboretum of the V.N. Sukachev Institute of Forest situated at the distance 38 km from Krasnoyarsk (Busim river basin). The study site is characterized by extremely continental climate with an average annual temperature 1, 3˚C, average winter precipitation is 80 mm, warm period precipitation is 350 mm, period of time without frost is 85 days, growth period is 180 days (Reference book on USSR climate 1967). The weather information provided by Archive of Krasnoyarsk Weather Service (http://rp5.ru) showed that in 2007 growth period (April-September) proved warmer (1,2-4,2˚C) than average and in 2008 April and May proved also warmer (1,8-2,6˚C) than average. In Arboretum there are soddy medium and strongly podzolic light loamy soils.

A four Picea A. Dietr. species from collection of Institute of Forest SB RAS Arboretum were studied. There are native species Siberian spruce P. obovata f. seminskiensis (Luchnik 1970), Black spruce P. mariana, which is transcontinental, occurring in boreal and temperate zone forests species (Viereck and Johnston 1990), Blue (Colorado) spruce P. pungens with natural range from Colorado to Wyoming at elevations of 1,800 to 3,400 m a.s.l. of the Rocky Mountains, USA (Daubenmire 1972), Glehn (Sakhalin) spruce – P. glehnii which distributed in Hokkaido, the southern Kuril Island, Sakhalin and Mt. Hayachine of Honsyu (Flora of Japan, 1995). All studied species are diploids with chromosome numbers 2n=24. Besides, one Siberian spruce tree with three B-chromosomes (2n=24+3B) were studied.

Seedling of P. pungens, P. glehnii and P. mariana were grown in 1966 from seeds obtained by seed exchange from native populations. The P. obovata f. seminskiensis seedlings were transferred to the Arboretum in 1972 from the forest nursery of M.A. Lisavenko Research Institute of Horticulture.
for Siberia (Barnaul, Altai) situated in a moderately continental climate (average annual temperature is 4.0°C). The age of sample trees is 48-50.

Branches with pollen cones from trees were collected. To analyze different stages of pollen development male strobili (microstrobili) were collected from the end of April till beginning of May. Material was fixed in the 3:1 ethanol: acid mixture in field conditions and from the cut branches in the laboratory. Material was stained with a 1 % acetoemiaxyalin or 2 % acetoamine for making a temporary squash slides. More than thousand of developing pollen grains were tested using light-optical microscope “MIKMED-6” (LOMO, Russia) and video visualization by DCM-500 microscope camera (SCOPETEK, China) with respect to stage of development and the occurrence and frequency of different types of irregularities.

The pollen collected from sample trees in the Arboretum was analyzed for morphology and size (in μm). Sizes of mature pollen grains (up to 30 per sample tree for each species) were measured. Occurrence and frequency of different types of pollen irregularities (up to 500-1000 pollen grains per sample tree) were calculated. The following parameters were measured: pollen body length (A), pollen body height (B), air wings length (C), and air wing height (D). The ratio B/A, which is characteristic of pollen body form also calculated (Monoszon-Smolina 1949). For these measures mature pollen collected at pollination time. The results were statistically analyzed using standard methods (Lakin 1990). Microsoft Excel was used to calculate descriptive statistics including the standard deviations and the confidence levels of the differences (Student-test). Growing degree-day values [GDD = (maximum temperature + minimum temperature) / 2) – 5] were calculated for each day summing from 1 March 2008 using weather archive data from Archive of Krasnoyarsk Weather Service (http://rp5.ru). If the result was negative for a day, no GDD were accumulated and a zero value was assigned for that day.

RESULTS

Pollen cones in sample trees enlarged in spring and developed during April–May. The rate of post-dormancy pollen cones development varied in different species depending on air temperature (Fig. 1). In native species P. obovata microspore tetrads observed at GDD about 0, 5˚C during the last ten days of April and buds swelled twenty days later at GDD 47, 3˚C. In all other species microspore tetrads observed on the 1-st of May, buds swelling occurred from the 17-th till 21-th of May at GDD 83, 3-113, 9˚. We presume there was a relationship between the climatic conditions and the pollen dispersal period. The rate of development of the post-dormancy pollen cone appeared to be strongly dependent on air temperature; it increased in warm weather and decreased when it became cool. It varied in spruce species from GDD 63,9˚ in P. obovata, till 113,9˚ in P. pungens, and 120,6˚ in P. glehnii and 192,2˚ in P. mariana.

Male reproductive development in conifers is initiated with the release of unicellular microspores from tetrads and includes several asymmetrical cell divisions (Singh 1978). There is the first and important stage of male gametophyte forming. Male gametophyte development showed a similar pattern across all four species. There is largely similar to other conifer species (Mergen and Lester 1961, Rozhdestvensky 1981, Gavrilov and Butorina 2005; Zhang et al. 2008, Bazhina et al. 2011, Bazhina 2014a, and others). Microspore tetrads were formed during 3-5 days (Fig. 2a). After tetrad forming microspores enlarged and pollen sacs were formed (Fig. 2b). After short period of dormancy the male gametophyte development was resumed. The haploid microspore cells (n=12) divided unequally twice, producing a small lens-shaped prothallial cell on the proximal side (opposite the wings) each time (Fig. 2c). The remaining large cell (antheridial initial) then divided unequally into a small generative and a large tube cell. Then generative cell divided again to form stalk cell and body cell. Most mature pollen grains of studied species had five cells: two degenerated prothallial cells, tube cell, stalk cell and body cell. However, in some of these were found that P. mariana pollen grains consisted of three protallial cells (Fig. 2d).

Pollen irregularities were found at pollen development of all species. Small size of pollen grain (SPG) with 1-2 air pollen sacs was the most common type of irregularities (Figs. 3e-f). Pollen body of SPG was in 1, 9-2,1 times less than the regular diameter (length and height), sizes of pollen sacs decreased in 1,4-1,6 times. The SPG could be well-developed as well as undeveloped. It was suggested that this type of irregularity
appeared to be meiotic irregularities when part of genetic material lost resulting from spindles damaged or chromosome mutations (Singhal et al. 2011, Rana et al. 2013). There was the only type of irregularities in *P. glehnii* and *P. obovata f. semikiensis* pollen (Table 1).

**Table 1—Pollen irregularities in *Picea* species at the V.N. Sukachev Institute of Forest Arboretum, %**

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency of irregularities, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>small pollen grains</td>
</tr>
<tr>
<td><em>P. obovata f. seminskiensis</em></td>
<td>0,2</td>
</tr>
<tr>
<td><em>P. pungens</em></td>
<td>13,9</td>
</tr>
<tr>
<td><em>P. glehnii</em></td>
<td>1,1</td>
</tr>
<tr>
<td><em>P. mariana</em></td>
<td>1,5</td>
</tr>
<tr>
<td><em>P. obovata</em> with B-chromosomes</td>
<td>27,8</td>
</tr>
</tbody>
</table>

Fig. 3. Pollen abnormalities in *Picea* species at the V.N. Sukachev Institute of Forest Arboretum: a – pollen grain with three pollen sacs; b - pollen grains with emergence; d - accreted sacs collar-shaped form; e, f - small pollen grains (SPG); g-l - accreting or gigantic pollen grains. Scalebars are 10 ¼m.

In *P. pungens* pollen part of SPG was achieved 13.9 %. Besides, pollen grains with 3-4 air sacs, accreted air sacs and lens-shaped pollen grains were also presented (Fig. 3a-d). In *P. mariana* SPG was only 1.5 % and pollen grains with 3-4 air sacs were also presented. The range of irregularities was wide enough in *P. obovata* with B-chromosomes.

**Table 2—Pollen size in *Picea* species at the V.N. Sukachev Institute of Forest Arboretum, µm**

<table>
<thead>
<tr>
<th></th>
<th>Pollen body</th>
<th>Pollen sacs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± S, µm</td>
<td>CV, %</td>
</tr>
<tr>
<td><em>P. obovata f. seminskiensis</em> **</td>
<td>74,7±1,26</td>
<td>9,2</td>
</tr>
<tr>
<td><em>P. obovata</em> with B-chromosomes</td>
<td>78,0±4,09</td>
<td>28,7</td>
</tr>
<tr>
<td><em>P. pungens</em></td>
<td>85,6±1,83</td>
<td>11,7</td>
</tr>
<tr>
<td><em>P. glehnii</em></td>
<td>63,4±0,60</td>
<td>5,1</td>
</tr>
<tr>
<td><em>P. mariana</em></td>
<td>53,6±0,31</td>
<td>6,4</td>
</tr>
</tbody>
</table>

* *The differences of pollen size between species were significant at p=0.05 except for pollen body (A) of *P. obovata* with B-chromosomes and *P. pungens*.

** *The differences of pollen size between *P. obovata f. seminskiensis* and *P. obovata* with B-chromosomes were not significant.*
Together with irregularities of general type the specific irregularities were observed (Figs. 3d, g-i). There were accreted or gigantic pollen grains (1, 5-2 times more in diameter) from two to four air sacs as well as without sacs and with accreted sacs collar-shaped form pollen grains. It is possible that gigantic pollen grains developed in result of irregularities in tetrad forming. According to Yu.F. Rozhdestvensky (1981), secondary fusion of microspore proplasts is also possible. Cell nucleus may fuse and result in diploid microspore formation or remain individual. In the latter case each nucleus develops into male gametophytes with common membrane.

Regular spruce pollen grains have, as a rule, two large symmetrical air sacs. *P. pungens* pollen was significantly greater than those of other spruce (Table 2, Fig. 3). The smallest pollen grains were formed in *P. mariana*. The measurements of pollen size were shown that pollen body form was varied from ball-shaped till ellipse-shaped. The ratio B/A was varied from 0,90 till 0,99 (*P. mariana*), and from 0,98 till 0,99 (*P. pungens*) and was made 0,86 (*P. obovata*), 0,81 (*P. glehnii*).

**DISCUSSION**

The development of the pollen grain in studied *Picea* species follows the same sequence as that described for *Pinus* by C. J. Chamberlain (1935), for some *Abies* species by F. Mergen and D.T. Lester (1961), J.N. Owens and M. Molder (1977), for *Picea glauca* and *P. sitchensis* by J.N. Owens and M. Molder (1979, 1980). The five-celled pollen grains are typical for *Picea obovata* and some *Abies* species (Mergen and Lester 1961, Nekrasova 1983, Owens and Molder 1977, Singh and Owens 1981). The study was revealed some features of pollen development at sample trees, such as variation in the number of protallial cells. As a rule, *Pinaceae* male gametophyte has two protallial cells (Hutchinson 1914, Tsiinger and Razmologov 1973, Fernando et al. 2010). However, in some *Pinaceae* species such as *Abies pindrow*, *A. sibirica*, *Pinus sylvestris*, *Picea excelsa*, *Larix leptolepis* some variation in the number of protallial cells was also found (Miyake 1903, Pollock 1906, Hutchinson 1914, Zhang et al. 2008, Bazhina et al. 2011). Moreover, there exists evidence that in *Abies balsamea*, *Picea excelsa*, and *Larix leptolepis* prothallial cells continue to divide in some and form di- or multi-nucleate cells.

Our study has shown that in addition to the normal pollen grains; small grains and grains with abnormal numbers of air sacs occurred. It was known that pollen heterogeneity by the size and shapes of pollen grains could result from some meiotic irregularities, such as chromatin stickiness and spindle irregularities (Singhal et al. 2011, Rana et al. 2013). When samplings obtained from natural populations are grown in botanical gardens and arboreta, changes in the natural-climatic conditions may provoke ecological stress and developmental anomalies especially at the beginning of reproductive phase, when spor- and gametogenesis become to fit new temperature and light regimes (Nekrasov 1971, Gavrilov and Butorina 2005, Bazhina et al. 2011).

Studies of Norway spruce (*Picea abies*) demonstrated that planting under non-adaptive conditions could induces a more rapid reproductive development at a shorter photoperiod than in the native environment (Owens et al. 2001). However, the low level of pollen abnormalities in most trees under observations could confirm a high adaptation capability in *Picea* species. The physiological features allow them to grow over a wider range of air temperatures than is reflected in its geographic distribution. The results obtained reveal the negative effects there is only in Blue spruce – *P. pungens*. Blue spruce grows in a climatic zone that is generally cool and humid with mean annual temperatures range from 3,9° to 6,1° C and mean minimum January temperatures range from 11,1° to 8,9° C (Daubenmire 1972; Fechner 1973). According to I.T. Kischenko (2011), in introduced *P. pungens* trees pollination occurs at GDD about 360°C. The considerable part of SPG could have resulted from the shock of moving trees from a uniform high to the lower ambient temperature of Siberian sharply continental climate.

The high level of pollen abnormalities may be also due to genetic features of the studied trees or mutations, since meiosis is under the genetic control (Khvostova and Yachevskaya 1975, Golubovskaya 1979, Bogdanov, 2003).
The greatest level of abnormal pollen was demonstrated \textit{P. obovata} tree with three B-chromosomes. Scientists recently consider that B-chromosomes maintain population polymorphism of many species in unfavorable natural conditions (Jones 1995, Muratova 2000, Kunakh 2010). Certainly, B-chromosomes appear resulting in main chromosome change ability and can influence adaptive plant potential, which becomes apparent not only by particular changes of plant phenotype with B-chromosomes but also with the increase of genome changeability level. This increases plant population polymorphism at unfavorable conditions of habitat.

Parameters of pollen size and form between species are due to genetics differences. There were differences between the species in all pollen size parameters in different populations (Nekrasova 1983, Lindbladh \textit{et al.} 2002; Major \textit{et al.} 2005). The pollen sizes within population range but pollen bodies form in populations close to ellipse-shaped. The index B/A was 0.76 and 0.89. The pollen size and form are affected pollen dispersal. The smaller black spruce pollen body in relation to sacs size probably makes the pollen lighter and increases its potential for dispersal, consistent with pioneer species ecology (Bazzaz 1979).

Generative organs of conifer, especially the male ones, are highly vulnerable to environmental factors. Climate fluctuations are regarded as the main factor responsible for decreased male reproductive capacity (Halterlein \textit{et al.} 1980, Maisonneuve 1983, Skroppa and Johnsen 1994). It is due to certain specificity of sexual reproduction that the spruce trees in the Arboretum are able to realize their high reproduction potential. In other words, this specificity causes such a high adaptation of the spruce reproduction system to the environmental conditions found in the Arboretum.

Recent studies have shown that different environmental factors may induce epigenetic changes in the mother trees, which changes are through the seeds to offspring and, can alter offspring gene activity by modifying its phenotype (Johnsen \textit{et al.} 1996, Herman and Sultan 2011). Conifer species display a wide phenotypic plasticity, making them masters in adaptation (Rohde and Junttila 2008). While the predicted climate changes, with increasing temperatures, might challenge their adaptive capabilities, conifers appear to have elaborated epigenetic regulatory mechanisms which can facilitate and retain changes in gene activities, enabling them to survive and reproduce successfully in changing environments (Yakovlev \textit{et al.} 2012).

Gamete formation at micro- and megasporogenesis as well as pollen viability is affect on seed quality. It have been shown (Arista and Talavera 1994, Owens \textit{et al.} 2001, Hak and Russel 2004) that deficiencies in high quality pollen at pollination time may be one of the principal factors responsible for the failure of seed gardens to produce sufficient quantities of viable seed. The investigations of features of spruce sexual reproduction in Arboret have contributed to science plant conservation.

**CONCLUSION**

Woody plants have developed adaptation mechanisms to modify their phenotype to tolerate changes in climatic conditions. The comparative analysis of pollen development of studied species demonstrates their high adaptive potential to severe environmental conditions. Monitoring ontogenesis of the spruce trees in a non-native climatic environment, such as in the Arboretum shows capability for adjustments to rapidly changing climatic regimes. Result of the studies can be use for studying plant response for climate change.

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