Arctic
Environmental
Research

Arctic Environmental Research 18(4): 162–174
UDC 581.162.41:582.477.6
DOI 10.3897/issn2541-8416.2018.18.4.162

Research Article

Pollination and pollen germination in common juniper (*Juniperus communis*: Cupressaceae)

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Academic editor: *Yuliya V. Bespalaya* ♦ **Received** 6 July 2018 ♦ **Accepted** 5 December 2018 ♦ **Published** 19 December 2018

Citation: Surso MV (2018) Pollination and pollen germination in common juniper (*Juniperus communis*: Cupressaceae). Arctic Environmental Research 18(4): 162–174. https://doi.org/10.3897/issn2541-8416.2018.18.4.162

Abstract

Pollination and fertilization are the most important and responsible stages in sexual reproduction of coniferous plants. The period of high concentrations of juniper pollen in overhead during pollination period is no more than 4–6 hours and within one calendar day. This dependence remains invariable from year to year, and does not depend neither from weather conditions, nor the peculiarities of the place where juniper grows. Influence of external agents on dynamics of exudation of a juniper pollination droplet is studied. The duration of exudation and volume of secretory liquid exuded by tissues of ovules during the period of prescription in the juniper depend from presence of external agents on a surface of pollination droplet, their sizes and physical and chemical properties. The pollination droplet chemical compound is studied. In sugars composition of juniper pollination droplet there are only two monosaccharides: glucose and galactose. the amino acids composition is prevailed by arginine, aspartic and glutamic acids. The results of the studies confirm complex chemical composition and multifunctionality of juniper pollination droplet. The pollination mechanism of juniper is effective and selective. The morphological structure of pollen grains of juniper predetermines the processes of pollen germination at early stages. The hydrophilic capsule, formed pollen hydration, promotes to exine rupture and shedding. This capsule remains until the fertilization. The distal tip of pollen tube remains in itduring all time of its growth. In culture *in vitro* development of pollen tubes of juniper proceeds non-uniformly. At definition of juniper pollen viability it is necessary to consider a stage of pollen tubes development.

Keywords

common juniper, pollination, pollination droplet, ovules, pollen grains, pollen tubes

Introduction

The extensive bibliography which is generalized in a number of reviews is devoted to the problem of pollination in Gymnosperms (Doyle 1945; McWilliam 1958; Owens et al. 1987, 1998; Anderson and Owens, 2000). The important role in pollination processes of the majority species in Cupressaceae, Taxaceae, Cephalotaxaceae families and many species in Podocarpaceae and Pinaceae families play the pollination droplet, more often accumulating on the top of the ovules in the reception period. The functional properties of pollination droplet of coniferous species were discussed in works of number of authors (Gelbart and von Aderkas 2002; Poulis et al. 2005; Mugnaini et al. 2007b; Wagner et al. 2007; Nepi et al. 2009).

The success of pollination in anemophilous coniferous species is caused by many factors. The most important of these factors are: the volumes of produced pollen, the terms of disclosing of a microsporangium, the duration of the period of pollen dispersion, the weather conditions during the pollination period, the time of days in which there is a mass departure of pollen and connected with it the ascending both descending streams of air and turbulence, aerodynamic properties of pollen grains, efficiency of mechanisms of pollen catching by receptive ovules. The least studied in this respect is juniper.

The pollen dispersion regime in natural and artificial populations of coniferous species has been studied, mainly, for some species of Pinaceae family (Willar et al. 1984; Nikkanen 2001; Nikkanen et al. 2002; Williams 2008). Only few works have been devoted to study the general dynamics of the pollen dispersion regime in Cupressaceae (Belmonte et al. 1999; Sabariego et al. 2012). Carrying out of these researches is in many caused by problems of clinical allergology and immunology (Altintaş et al. 2004; Diaz de la Guardia et al. 2006; Ianovici et al. 2013). Recently, these researches more and more associated with problems of long distance migration of pollen mass (Rogers and Levetin 1998; Necib and Boughediri 2016; Puljak et al. 2016), and evolution of pollination (Friedman and Barrett 2009).

Evolution of pollination mechanism in Gymnosperms went from entomo- to anemophily (Labandeira et al. 2007). It has left traces on a structure and functional features of tissues of ovules and pollen grains of conifers. The common juniper is obligatory diecious species. Like most coniferous plants, the common juniper is anemophilous species, although it seems that cases of entomophilies are not excluded for it.

Morphology of pollen grains of species of the Cupressaceae family and *Juniperus* genus is considered in the works of many authors (Southworth 1986; Bortenschlager 1990; Kurmann 1994). The mechanism of exine rupture and shedding at pollen germination, peculiarities of growth and development of pollen tubes and microgametogenesis in juniper are considered in the works of E. Duhoux (1972a, b, 1974, 1982); Fernando et al. (2005); Takaso and Owens (2008).

The purpose of the research was to study the processes of pollination and biology of pollen germination in common juniper.

Materials and methods

Collection of microstrobiles and pollen extraction

Branches with mature microstrobiles were cut off from male juniper plants prior to the beginning of microsporangium disclosing. Branches with microstrobiles were located in vessels filling with water, which were put on newsprint sheets so that, whenever possible, the most part of pollen after pouring has appeared on a paper. Pollen was sifted through small sieve and stored in the glass test tubes; leaky closed by wadded stoppers, in desiccators containing calcium chloride, in the refrigerator at temperature +1 … +2 °C.

Scanning electronic microscopy

Electronic microscopy studying of pollen grains was making by means of scanning electronic micro-

scope Sigma-Zeiss. Water satiated pollen was freeze dried up and, after a gold dusting, scanned at various modes of increase.

Pollen tubes growth

Pollen incubated *in vitro* in "damp chambers" in Petri cups at +26.5°C on 1.0 % th agar medium with addition 5 %-s' sucrose. Duration of growing depended from speed (intensity) of fungal mycelium growth. The percentage parity of pollen grains on following categories was defined: 1 – pollen grains have not germinated; 2 – pollen grains have formed hydrophilic capsule and shedded its exine, microspore has no turned into microgametophyte and remains in the centre of hydrophilic capsule; 3 – microspore has turned in the two-cellular microgametophyte, the formed pollen tube oval or "shoelike" forms completely, or nearly so entirely is inside of hydrophilic capsules; 4 – pollen tube was generated, her proximal tip left from capsules, but her distal tip remains inside of hydrophilic capsules. The average length of a pollen tube was defined be means of measuring of 100 casually taken pollen tubes of 4th category or directly on the computer monitor, or with the aid of ocular-micrometer. For supervision over divisions and moving of nuclear structures in growing pollen tubes it was made pollen incubation in the distilled water by means of "hanging drop» method. Pollen tubes were not stained, or stained by the various dyes which choice depended from the purposes of researches.

Dynamics of pollen dispersion

For studying the dynamics of a pollen dispersion in natural populations of a juniper were used "pollen traps", structurally representing a week clockwork with a rotating drum. On a drum the transparent polyethylene film with the put thin layer of vaseline was imposed. The drum was tightly closed by a box from thin plexiglas. On the forward party of a box the vertical crack in width of 1.2 mm for pollen catching became. The "pollen traps" worked by a weather-vane principle, i.e. the reception crack always settled down towards to a horizontal stream of air. Traps were established on poles in height of 1.3 m near to pollen modulators.

Simultaneously with installation of traps near to them the week thermographs working synchronously with traps were established. After shooting of a registering film concentration of pollen in air on each interval of time was defined by a method of light microscopy by means of a film-substrate divided into squares 1x1 mm. Thus, a continuous time number (dynamics) of density of a pollen cloudlet in the set point turned out.

Dynamics of ovular secret exudation

Studying of influence of external agents on dynamics of exudation and retraction of pollination droplets of juniper was the purpose of experiments. Branches in length of 50–70 cm with receptive macrostrobiles were cut off from female plants, but before the disclosing of microsporangium at the male plants. Branches kept within cuts downwards damp sphagnum in leaky packed and tightly closed polyethylene packages. Within 3 hours they were delivered in laboratory. For experiences shorter were used (15–30 cm) the top pieces which were put in vessels with water. During experiences water was added as required. Measurements of diameter of droplets made perpendicularly to axes of micropylar canal by means of a measuring scale of binocular microscope MBS-10 (Russia). In experience of 2015 tested 3 variants of pollination: pollination by juniper pollen, pollination by pollen of a Scots pine, without pollination. In experience of 2016 tested 5 variants of pollination: pollination by juniper pollen, pollination by silica gels with diameter of particles 25–40, 63–100 and 160–200 μ, without pollination. Pollen and silica gels were put on a surface of droplets by dispersion from a short distance with the help preparations needles. Results of pollination were supervised visually. Measurements of diameter of droplets in experiences made with periodicity of 4–30 hours from the moment of its occurrence to a total disappearance.

Chemical compound of pollination droplet

For chemical compound studying the secretory liquid accumulating in the form of droplets on tips of ovules in receptation period took by means of narrow strips of a filtering paper. Strips before using stored in tightly closed plastic test tubes in the refrigerator at -20 °C. Qualitative structure of flying fractions of organic compounds in pollination droplet of juniper droplet determined by means of gas chromato-mass spectrometer QP-2010 Ultra (Shimadzu, Japan). Amino acids compound of pollination droplet and pollen of juniper defined with the help of amino acid analyzer BioChrom 30+ (Biochrom, Great Britain). Sugars compound of pollination droplet and juniper pollen studied by method of high-performance liquid chromatography with using of HPLC-system Nexera XR (Shimadzu, Japan).

Light microscopy

Macrostrobiles in receptation period and soon after him were fixed in acetic alcohol. All fixed material washed out and stored before use in 70 %-s' ethanol. Histological mounts in the thickness 8–10 μ were stained by methyl green – pyronine G. Viewing and photographing of permanent microtomic preparations carried out by means of laboratory microscope AxioScope A1 (Zeiss) complete with digital camera Canon G10. Editing of images made by means of license software AxioVision LE Release 4.8.1. Preparation of ovules made under binocular microscope MBS-10. Temporary preparations stained in 0.25 %-s' water solution of safranine. Photographing and editing of images made by means of video ocular Pro-MicroScan 5888 and license software Scope-Photo 3.0.

Results

Pollen dispersion regime in natural populations of a juniper studied in northern taiga (Arkhangelsk region) in 2007–2016. This regime in juniper populations differs from the general scheme of a pollen dispersion regime in populations of main forestry coniferous species at which the total duration of the pollination period can be stretched for some weeks and for which the sinusoidal type of a curve of distribution of density of a pollen stream with maxima in midday and minima at night is characteristic. In juniper such curve is presented by only one maximum within one calendar days at the expense of very short (no more than 4–6 hours) the period of intensive pollen dispersion which is usually coincided with midday o'clock. During this short period dispersed from 90 to 98 % from pollen total (Fig. 1).

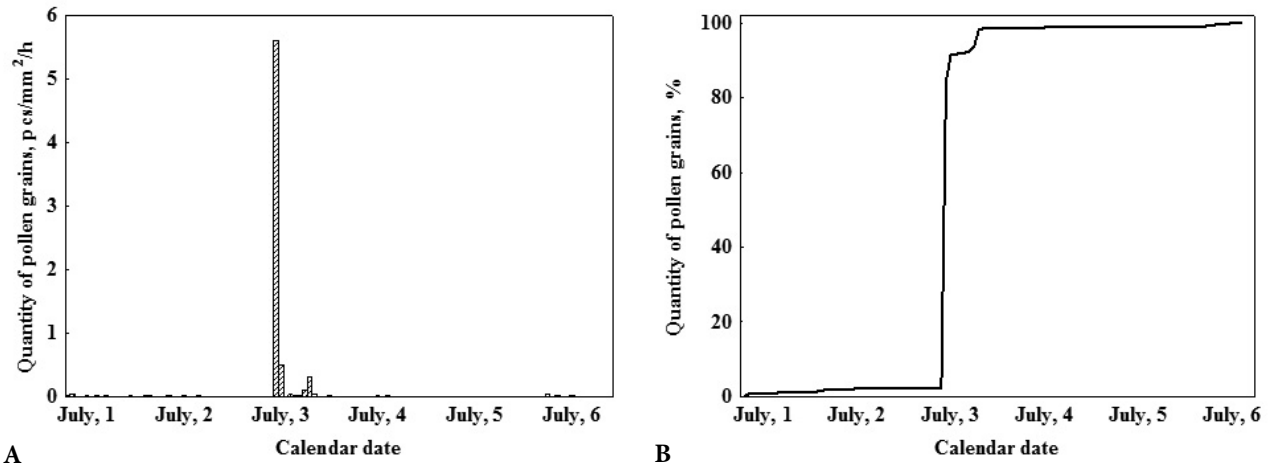

Fig. 1. Pollination regime in a natural population of a juniper in 2007. **A** – histogram, **B** – accumulation

This regularity remains from year to year, and does not depend neither from weather conditions in a receptation period, nor from features of a place of growth of a juniper.

Pollen efficiency of male plants of the juniper growing in compact biogroups, on well shined slopes, high enough, also makes 10^{11}–10^{13} pollen grains on 1 hectare. It is comparable to pollen efficiency of such coniferous species, as a spruce (10^{12}–10^{13} pieces / hectare) and a pine (10^{13}–10^{14} pieces / hectare). The aerodynamic characteristics juniper pollen comes nearer to those species the pollen grains of which have air sacs.

Consequently, such short period of mass pollen dispersion in juniper it is possible to explain only to that its scattering pollen starts from very small height.

Therefore almost all of made pollen settles nearly from the modulator. Carrying over some significant volumes of pollen on the long distances in juniper is improbable owing to the same circumstance.

Up the time of pollination the juniper ovules are differentiated on integument and nucellus (Figure 2B).

In chalazal part of nucellus accompanied by cells of nucellar tapetum one or several mother's cells of macrospores stand apart. In a receptation period the micropylar channel serves for lifting secretory liquid to the top tips of the integuments of ovule. Pollen is caught by a pollination droplet, sticking to its surface (Fig. 2A).

Retraction of secretory liquids provides a translocation of pollen grains from micropylar tips of integument to nucellus top where pollen grains shaded exine and germinated in pollen tubes. Right after the pollination the micropylar channel of the ovule in subnucellar zone almost is completely closed owing to cross-section elongation the cells composing internal surfaces of integuments (Fig. 2C).

Very narrow aperture in micropylar channel remains for the winter. Definitive isolation of megasporangium occurs the next year after the beginning of formation of "cone-berry" when cells of internal surfaces of adjacent megasporophylls are closed in proximal parts by means of gear "lock".

Important role in the course of pollination (catching of pollen grains and its translocation to the top of nucellus) in *Juniperus* plays pollination droplet accumulating on the tips of integuments in receptation period. J. Owens et al. (1998) believe that pollination droplet of the majority coniferous species

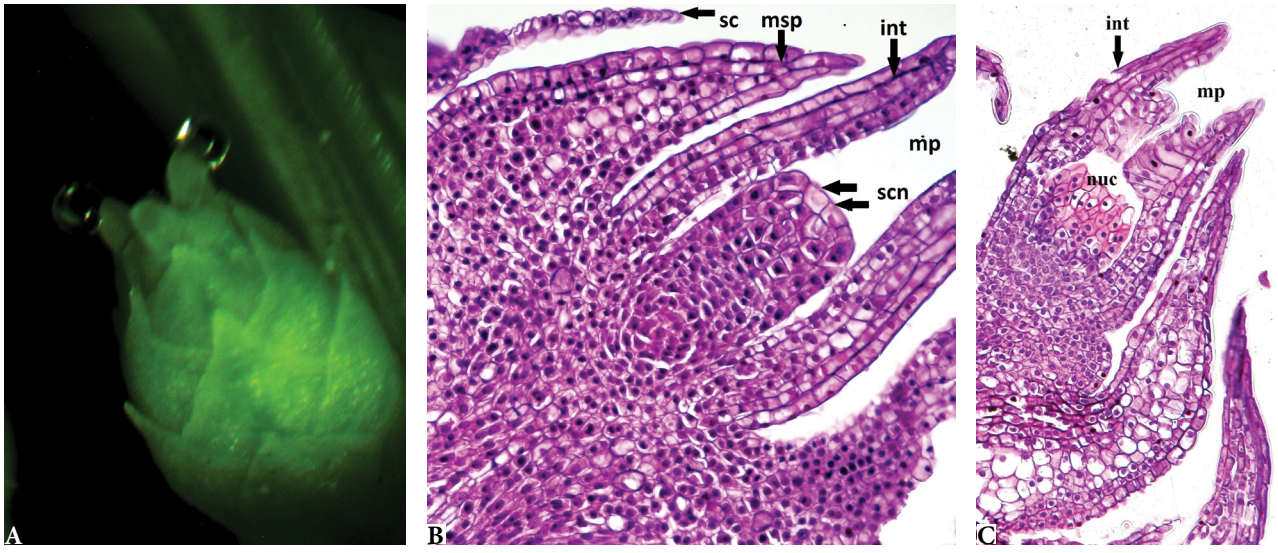

Fig. 2. Juniper´s ovules directly ahead of (**A** and **B**) and soon after (**C**) pollinations (microtome cuts are stained by methyl green - pyronine G). int – integument, nuc – nucellus, mp – micropyle, sc – integumentary scales of ovule, msp – megasporophyll, scn – secretory cells of nucellus

are secreted by apical cells of nucellus, but, probably, also the cells of tissues of a megagametophyte and integument. In juniper, in our opinion, most likely, the secret is produced by several cells of apical zone of nucellus. These cells can be identified by nucleus absence in them (Figure 2B, C).

After pollination these cells are lysed, forming poroid on apical part of nucellus. Products of this catabolism are, possibly, a nutrient medium for the growing pollen tubes which are carrying out heterotrophic type of a feeding, at least, at initial stages of its development.

The pollination droplet in juniper is multifunctional. The ovular secret serves not only for catching of pollen grains, but also participates in cognizance processes, prevents premature of exine rupture and shedding, delays development of micro flora and promotes growth and development of pollen tubes at initial stages of pollen germination. The pollination droplet reacts to external irritants. This reaction is selective.

After pollination of receptive ovules by pollen of juniper the full retraction of exudation secret has occur to the end less than for 4 hours. After pollination by alien pollen the full retraction of pollination droplets inside ovule has occurred only 12 hours later. In the absence of pollination the exudation activity of apical zones of the nucellus proceeded not less than 60 hours. The sizes of droplets have thus increased to maximum, on the average, by 40 %. Frequent cases of merging of droplets of two or all three ovules in one general for a macrostrobile a large droplet were observed.

In 90 hours on tips of the majority of ovules already enough small droplets were observed, many ovules were without droplets, large droplets have remained only on individual ovules. In 120 hours at all not pollinated ovules has occurred full retraction of secretory liquids.

Experiences on artificial pollination of secretory droplets by the silica gels with particles of various diameters have shown that their size influences on duration of exudation and speed of retraction of secretory liquids. The more largely a particle of silica gel, the retraction of pollination droplets is longer. Large particles (diameter more than 40 μ) cannot get in narrow micropylar channel of ovule and accumulate at its input outside. Particles of silica gel, relatives in the sizes to juniper pollen grains, free get in an ovule, being involved there together with secretory liquid.

The cited data confirms results of experiments of S. Mugnaini et al. (2007a) on artificial pollination of secretory droplets by silica gels with the sizes of particles of different diameter. In the researches these authors also have proved influence of the size of particles of silica gels on duration of exudation and speed of retraction a secret of receptive ovules of juniper.

As a part of flying fractions of organic substances pollination droplets of juniper it is revealed about 40 substances belonging to various classes. The percentage parity of these fractions makes: alkanes – 33.79 %, monoterpenes – 0.29 %, triterpenes – 16.97 %, sesquiterpene alcohols – 1.33 %, eteri composti –18.41 %, carbonic acids – 1.81 %, amides of carbonic acids – 8.81 % (Table 1).

It is necessary to note the high maintenance of squalene (substance of triterpenes serie belonging to group carotenoids') – 14.85 %, and also eteri composti, including aethers of phthalic (12.07 %) and trifluoroacetic (belonging to fluorochrome's – 8.23 %) acids.

Into the sugars compound of pollination droplets of juniper only two monosaccharide's: glucose (65.8 %) and galactose (34.2 %) are included (Table 2).

The analysis of amino acids compound of pollination droplets has shown that in it prevail arginine (25.5 %), asparagine acid (20.7 %) and glutamine acid (15.1 %). Presence cysteine (3.8 %), glycine (1.9 %), histidine (2.8 %), leucine (7.5 %), lysine (2.8 %), methionine (2.8 %), proline (3.8 %), serine (7.6 %) and valine (5.7 %) is noted also (Table 3).

However the general maintenance of amino acids, as well as sugars, in secretory droplet more poorly and 10 times less in comparison with pollen.

It is necessary to notice that researches of chemical compound of pollination droplets in Gymnosperms it is not enough, and it, unlike nectar floral plants (Dumas et al. 1988), still remains studied insufficiently full. It is known about presence in it mono- and disaccharides, amino acids, organic acids (Nepi et al. 2009). Proteom-

Table 1. Structure of flying fractions of organic substances in pollination droplet of juniper

Peak	Name	Height, %	Peak	Name	Height, %
1	4-Penten-2-ol	0.38	22	Tetracosane	3.27
2	2-Hexene, 2.5.5-Trimethyl-	0.37	23	Cyclononasiloxane, Octadecamethyl-	0.82
3	Alpha.-Pinene, (-)-	0.29	24	not identified	1.74
4	3-Cyclohexen-1-ol, 4-Methyl-1-(1-methylethyl)-	0.33	25	Pentacosane	4.07
5	Decanal	0.17	26	Docosanoic acid, methyl ester	1.81
6	Benzoic acid, 4-ethoxy-, ethyl ester	0.27	27	Bis(2-ethylhexyl) phthalate	6.16
7	endo-1-bourbonanol	0.55	28	Tetracosamethyl-cyclododecasioxane	1.39
8	.alpha.-Cadinol	0.75	29	Hexacosane	4.07
9	.alpha.-Bisabolol	0.58	30	Hexacosyl acetate	0.75
10	Heptadecane	0.41	31	not identified	0.77
11	Heptadecane, 8-methyl-	0.39	32	not identified	1.47
12	1.2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.21	33	Heptacosane	4.00
13	Nonadecane	0.56	34	Tetracosanoic acid, methyl ester	4.00
14	Dibutyl phthalate	5.91	35	Octacosane	3.12
15	Eicosane	1.11	36	Squalene	16.97
16	Heneicosane	1.69	37	Cyclononasiloxane, octadecamethyl-	1.53
17	Docosane	2.95	38	Nonacosane	2.51
18	Tricosane	3.13	39	Cyclononasiloxane, octadecamethyl-	0.98
19	9-Octadecenamide	6.41	40	Tetratetracontane	1.18
20	9-Octadecenamide	1.71	41	Hexatriacontane	1.33
21	Octadecanamide	0.69	42	Octatriacontyl trifluoroacetate	8.23

Table 2. Compound and the relative quantity of sugars in pollen and ovular secret of common juniper

Sample	Quantity of sugars (in numerator - mcg/ml, in denominator – %)			
	arabinose	galactose	xylose	glucose
Pollen				
	206.888	562.498	13.651	210.748
	20.8	56.6	1.4	21.2
Droplet				
	0	4.860	0	9.366
	0	34.2	0	65.8

ic researches testify to presence at it of albumens the majority from which is not identified (O'Leary 1998; Poulis et al. 2005; Wagner et al. 2007). The complex chemical compound of pollination droplets testifies to its multifunctionality.

Features of a structure and biometric parameters of pollen grains define their aerodynamic properties (Schwendemann et al. 2007). Pollen grains of juniper are one-celled and, actually, are the microspores which have not germinated in a microgametophyte. Pollen grains have almost correct spherical form and are deprived air sacs. Pollen grains have one functional pore (distal aperture) which at dry pollen is covered by a lidlet (Fig. 3A). The surface of sexine is small granulated, and covered by numerous orbicules which have 450–650 nanometers in diameter

Table 3. Compound and the relative quantity of amino acids in pollen and ovular secret of common juniper

Amino acid	Pollen, % from total	Droplet	
		% from total	comparatively to its quantity in pollen, %
L-Alanine	5.9	0	–
L-Arginine	3.8	25.5	0.71
L-Aspartic acid	13.7	20.7	0.16
L-Cysteine	0.7	3.8	0.57
L-Glutamic acid	17.9	15.1	0.09
Glycine	5.7	1.9	0.04
L-Histidine	2.5	2.8	0.12
L-iso- Leucine	4.2	0	–
L-Leucine	7.5	7.5	0.11
L-Lysine	7.6	2.8	0.04
L-Methionine	1.0	2.8	0.3
L-Phenylalanine	3.9	0	–
L-Proline	2.7	3.8	0.15
L-Serine	7.3	7.6	0.11
L-Threonine	6.6	0	–
L-Tyrosine	3.8	0	–
L-Valine	5.2	5.7	0.12

(Fig. 3). Orbicules are supplied by small barblets with which help they are unsteadily kept on a pollen grain surface (Fig. 3E). At hydration of pollen grains the part of orbicules comes off, forming perforations on exine surface (Fig. 3D).

The sizes of pollen grains of a juniper make, on the average, 25–26 μ, and have very low individual and intra-population variability (CV=2.4–2.5 %). Distribution of pollen grains in the sizes comes nearer to a normal curve. In common juniper growing in northern taiga as anomalies the dwarfish and deformed pollen grains are noted only and, extremely seldom, there are meet huge polyploid grains. The total of anomalies does not exceed 0.1 %.

Discussion

The first metamorphoses of pollen grain of a juniper after sowing on a nutrient medium are result from pollen hydration. These metamorphoses are not connect-

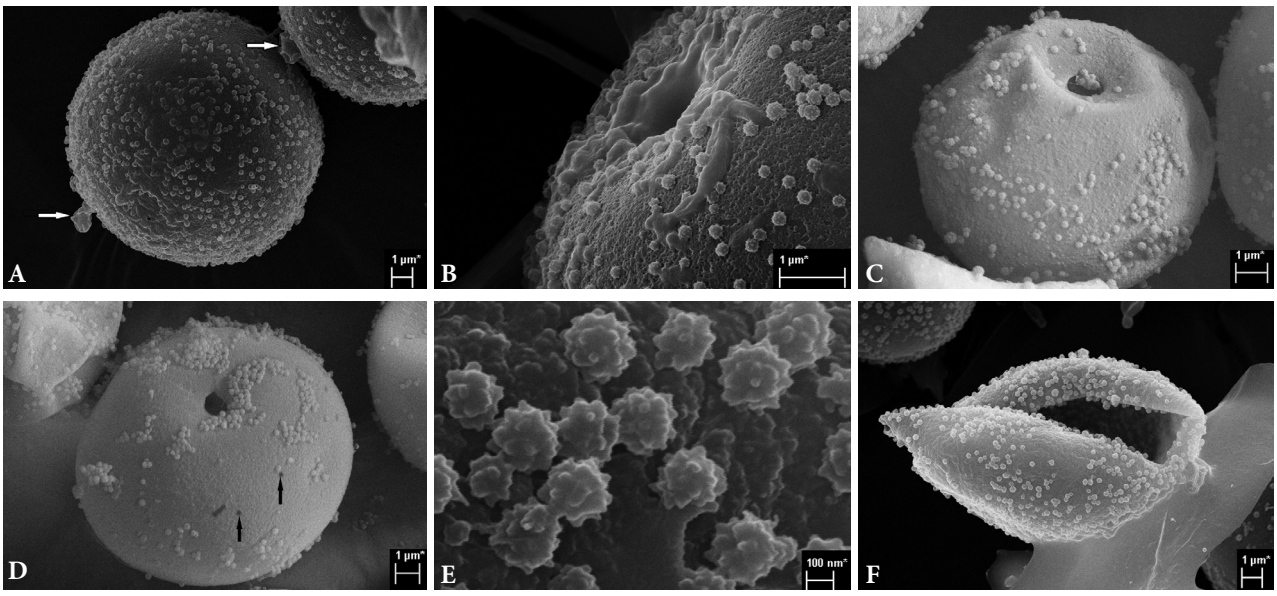

Fig. 3. General view and exine surface of juniper pollen grains. **A** – the lidlets closing a functional pore (shown by arrows); **B** – functional pore and orbicules on exine surface; **C** – aggregation of orbicules in zone of functional pore; **D** – perforations, formed on exine surface of pollen grain as a result of orbicules come off (shown by arrows), the shade from one of orbicule loused contact with sexine is visible; **E** – orbicules on exine surface are supplied with barblets; **F** – shedded exine (on an external surface of endonexine appear through orbicules, attached to an external surface of sexine)

ed with pollen grain germination to pollen tube, and caused by exine rupture and shedding. The detailed description of this process has been given for the first time by E. Duhoux (1982). Only small specifications and additions to this research are more lowly given.

Procedure of exine rupture and shedding proceeds rather dynamically.

At hydration of pollen the water is soaked up by an osmotic way through a functional pore internally to pollen grain. Between an internal surface of endonexine and internal intine of microspores (the future wall of a pollen tube) the ring (a water layer) is formed. The external border of this ring is delimited from an internal surface of endonexine by an elementary semipermeable membrane. As a result is formed a hydrophilic capsule which very quickly increases in volume and which with escalating effort the wall presses on a pollen grain cover. Under growing turgor the walls of quickly increasing her volume hydrophilic capsules the exine of pollen grain it is opened with two equal valves, and it is shaded (Figure 4A).

The hydrophilic capsule starts to leave through a functional pore and after shutters of a cover of pollen grain will be moved apart widely enough, exine very quickly climb down a hydrophilic capsules. Diameter of distal aperture of pollen grain of a juniper is much less than diameter of hydrophilic capsule with being within it microspore. Exine of juniper

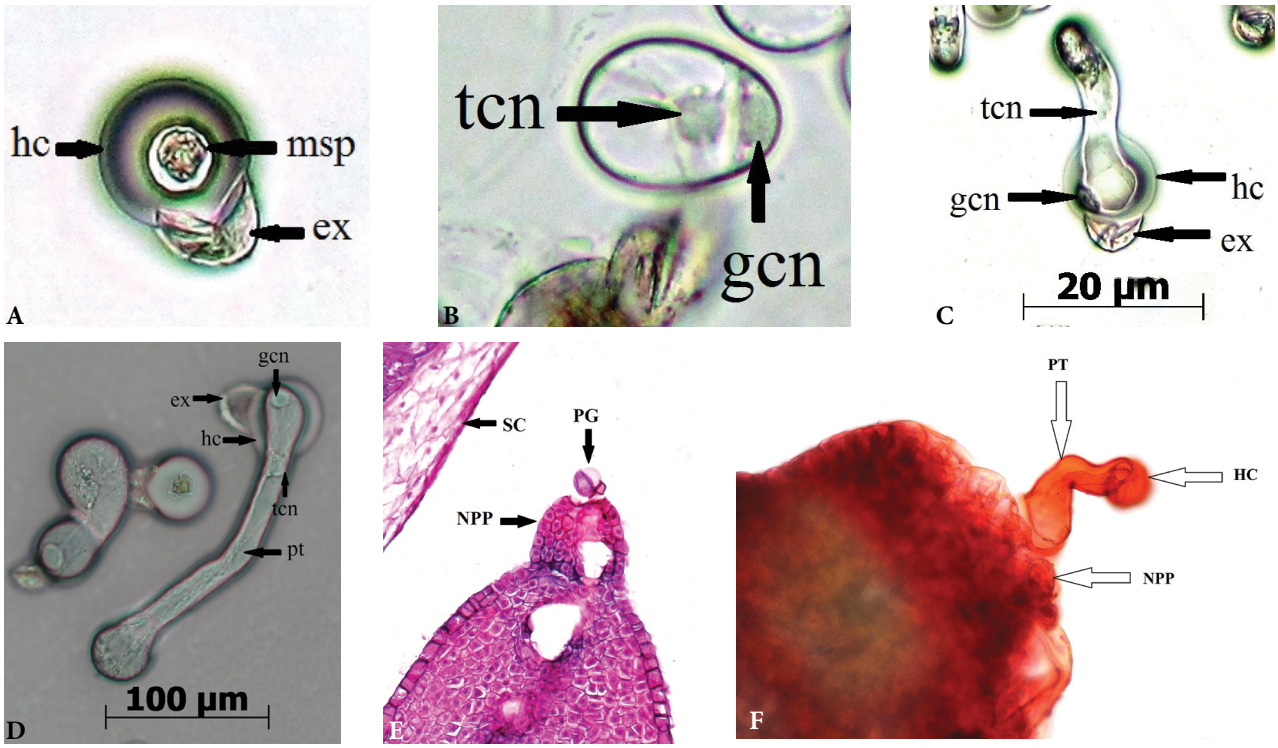

Fig. 4. Pollen germination of common juniper *in vitro* (**A**–**D**) and *in vivo* (**E** and **F**): **A** – formation of hydrophilic capsules and shedding of exine of pollen grain (stage 1); **B** – two-cellular microgametophyte (stage 2); **C** – pollen tube on stage 3 (it is visible shedding exine, tip of a pollen tube leaves from hydrophilic capsules, without breaking through its wall); **D** – generated pollen tube, hydrophilic capsule has remained; **E** – pollen grain on a surface of a pollen pillow of nucellus (exine it was opened with two equal valves, is visible not germinated microspore); **F** – distal tip of a pollen tube shortly before fertilization (later 13 months after pollination, remained hydrophilic capsule is visible). ex – exine; hc – hydrophilic capsule; msp – microspore; gcn – nucleus of generative cell; tcn – nucleus of tube cell; pt – pollen tube; npp – pollen pillow of nucellus; pg – pollen grain; sc – seed coat

pollen grain thick enough and, apparently, strong enough. Possibly, rupture of exine is in many respects caused by perforations presence, appearing in attachment places of orbicules after pollen grain hydration. Localisation of orbicules on a surface of exine is deprived any orderliness. However, as a hydrophilic capsule represents a sphere of almost ideal form, pressure of its walls uniform on all area of an internal surface of endonexine. Therefore, rupture of exine occurs in area of distal apertures, thus edges of the formed valves turn out ideally equal. That hydrophilic capsule has own external wall in the form of a membrane, proves to be true the results of staining of juniper pollen grains after its hydration. Accurate external contours of a capsule are shown at staining by water solutions of a rose bengal, aceto-iron-hematoxylin – chloral-hydrate, iodine – potassium iodide – chloral-hydrate, congo red, safranin, chromic dark blue, eriochrome black and of some other staining. Character of fluorescence of hydrating juniper pollen grains at staining by acridine orange (fluorescence conditions: the operating mode 20 Rhod, emission 575–640 nanometers) testifies that capsule contents represents weak electrolyte of an uncertain chemical compound. To the pollen germination and microgametogenesis in vitro in common juniper a series of articles of E. Duhoux (1972b; 1974) is devoted. Our supervision serves as addition to this research.

Germination of microspores into a microgametophyte begins still inside hydrophilic capsules, and is connected with prophase of the first mitosis.

After formation of a two-cellular microgametophyte its gradual transformation into a pollen tube is begins which gets at first oval, then "shoelike" forms, but still entirely holds in hydrophilic capsule (Figure 4B).

The generative cell remains in distal part of pollen tube, nestling on one of its lateral walls, and nucleus of vegetative cell (cells-tubes) gradually moves more close to its central zone. Gradually the growing tip of a pollen tube reaches the border of hydrophilic capsules and leaves it, without breaking through its wall (Figure 4C).

In vitro the hydrophilic capsule remains during all time of pollen germinating. At germination of juniper pollen *in vivo* the hydrophilic capsule remains during all the period of pollen tubes growth, up to the fertilization (Figure 4E).

Pollen was incubated on agar medium with sucrose addition (A, C, D) and in dH_2O by method of "a trailing drop" (B). A microtome preparation of an ovule, staining by methyl green – pyronine G (E). Ovules were prepared and then stained by safranine (F).

Probably, thereby it is prevented the drying of distal tip of a growing pollen tube. The pollen pillow starts to be formed from apical cells of a nucellus after growing of a pollen tube into megasporangium tissues, thus the proximal tip of a growing pollen tube always is behind the bottom border of a pollen pillow.

With the beginning of growth of a pollen tube around of a vegetative nucleus the intensive synthesis of polysaccharides and lipids which concentrate nearby proximal tip of tube is carried out. Cytoplasm in distal parts of tube keeps an optical transparency, in distal tip of tube there is also a generative cell of a microgametophyte.

Incubation of the juniper pollen *in vitro* does not represent essential experimental difficulties. For incubation of juniper pollen are optimums the agar mediums containing sucrose.

At the incubation of the juniper pollen in dH_2O, in water solutions of sucrose or mineral salts the development of a pollen tube comes to the end at a "shoe" stage more often. On agar mediums containing sucrose the duration of growth of pollen tubes is limited only of dynamics of developments of micro flora, to avoid which difficult owing to natural background of contamination of pollen with mold fungi spores. At the prolonged incubation period the long (more than 200 μ), massive enough pollen tubes, frequently with clublike thickenings of proximal tips are formed that testifies to their high energy of growth.

For definition of indicators of viability of juniper pollen the terms of incubation *in vitro* should be not less than 6–7 days.

It is necessary to notice that there are no the accurate criteria, it is necessary to consider what pollen germinated and what – is not present. To state an exact estimation of pollen viability at species with the long period of formation of pollen tubes and with non-uniform germination it is inconvenient enough.

The juniper pollen viability, in our opinion, can be estimated approximately as the sum of all pollen grains which have generated high-grade pollen tubes with length of 3–4 diameters of pollen grain (stage 3), half of two-cellular oval or "shoelike" gametophytes (stage 2) and quarters of all pollen grains which have shedded exine, but not generated two-cellular gametophytes (stage 1), expressed in percentage of total of the seen pollen grains:

$$L = 0.25L_1 + 0.5L_2 + L_3 ,$$

where L – pollen viability, %; L_1, L_2 and L_3 – stages of pollen germination.

Individual variability of indicators of pollen viability is always high or very high. The minimum volume of excerpts from investigated sample for definition of pollen viability at level of probability of faultless judgments P=0.95 in case of the greatest possible variability of a sign (L=50 %) is equal to 400th pollen grains.

Conclusions

The purpose of researches was studying of processes of pollination and biology of germination of pollen in common juniper. It is established that the pollination regime in natural populations of this species is characterized by very short (no more than 4–6 hours) period of mass pollen dispersion. The important role in the course of pollination in *Juniperus communis* is played the pollination droplet accumulating on a top of ovule's micropyle in receptation time. Experiences on artificial pollination have shown that pollination droplet actively reacts to external irritants: duration of exudation and retraction, and also quantity of secreted of ovular liquid depends from presence or absence on a surface of a droplet the particles, their sizes and, obviously, their physical and chemical properties. The conducted researches have confirmed a complex chemical compound of pollination droplets in juniper that is indirect acknowledgement of its multifunctional. Functional properties and chemical compound of pollination droplets deserve deeper studying. The processes of hydration previous to germination of pollen are predetermined by morphological features of pollen grain of a juniper. Experiments on pollen incubation *in vitro* and supervision over development of pollen tubes *in vivo* have shown that the important role in processes of exine rupture and shedding and the subsequent development of a pollen tube in common juniper is played hydrophilic capsule remaining during all life of pollen tubes, up to the fertilization. It is established that for juniper pollen incubation are optimum agar mediums containing sucrose. By results of these experiments the scheme of calculation of pollen viability of a juniper at incubation *in vitro* is offered.

Acknowledgements

The reported study was funded by RFBR according to the research project № 18-04-00056

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