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OPTICS OF CLUSTERS, AEROSOLS, AND HYDROSOLES

Cluster Composition of Anemophilous Plant Pollen Entering the Atmosphere

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Abstract—Results of studying pollen emission into the atmosphere are presented for 26 anemophilous and three entomophilous plant species for which optional anemophily is possible. The percentage of clusters of two or more pollen grains of the total number of pollen particles entering the atmosphere is estimated. It is shown that such clusters were formed in significant quantities in all series of experiments. The percentage of pollen clusters reaches ~71% of the total number of pollen particles. The percentage of pollen grains in the composition of the clusters reaches ~94% of the total number of pollen grains.

Keywords: pollen, anemophilous plant, atmospheric aerosol, cluster

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INTRODUCTION

Pollen of anemophilous plants is an indispensable component of the coarse fraction of atmospheric aerosol. Its propagation in the atmosphere is a physical process necessary for seed reproduction of anemophilous plants, the main producers of extratropical land biomass. Pollen grains (PGs) cause outbreaks of allergic reactions in 30% of the population [1] and take part in the transfer of chemical elements in biocenoses.

The high allergenicity of pollen has led to the creation of many aero-palynological monitoring stations throughout the world. They are often combined into regional and national networks where long-term observations are carried out by standard techniques. In 2016, there were 879 stations of pollen aerosol sampling in the world: 9 in Africa, 151 in America, 182 in Asia (143 in Japan), 525 in Europe, and 12 in Oceania [2]. In Russia, there are nine stations: in Moscow, St. Petersburg, Yekaterinburg, Krasnodar, Perm, Rostov-on-Don, Ryazan, Stavropol, and Tyumen (https://allergotop.com).

Most often, pollen concentration in the atmosphere is determined using Hirst, Burkard, and Lanzoni slit aspiration traps (>600, or 70% of the stations). Features of pollen particle (PP) morphology (large size, the mean diameter of PG of anemophilous plants is 20–40 μm, drying deformation, and the presence of clusters) are caused by significant difficulties in the process of pollen aerosol (PA) entrapment. The sampling is nonisokinetic, PP settling on the walls of collecting devices is observed [3, 4], the sampling is accompanied by destruction of clusters [5], and the efficiency of trapping of >10-μm particles is unsatisfactory [6] (for example, ~15% of cereal pollen is not trapped). The analysis of samples collected by impactors requires qualified executives and takes from three to ten days. The error of pollen concentration measurements in air is ~30% [7]. The currently available technique of PA sampling did not provide continuous monitoring during the COVID-19 pandemic [8]. The quarantine and isolation of personnel prevented observations from being carried out. It is also necessary to note that collections of PA samples allow one to judge the pollen content in the atmosphere only in the vicinity of an observation site. Even in Europe (for example, in Bavaria) the number of pollen monitoring stations is insufficient for forecasting the pollen transfer in the atmosphere [2].

Currently, the AutoPollen program is operative in Europe. It is aimed at creating a prototype of a network of fully automatic pollen monitoring stations. To cover main bioclimatic zones of Europe, it is planned to additionally deploy 200–300 automatic stations allowing one to obtain data in the real time mode (several minutes after sampling). It is believed that timely acquisition of information about taxonomic affiliation, terms of entering, and concentration of PPs in the atmosphere will notably reduce the direct and indirect health costs associated with allergy, currently estimated between €50–150 billion/year [8].

The area of Russia, three times the area of Europe, with a much lower population density and an extremely uneven distribution of the population, objectively hinders the creation of a network of pollen

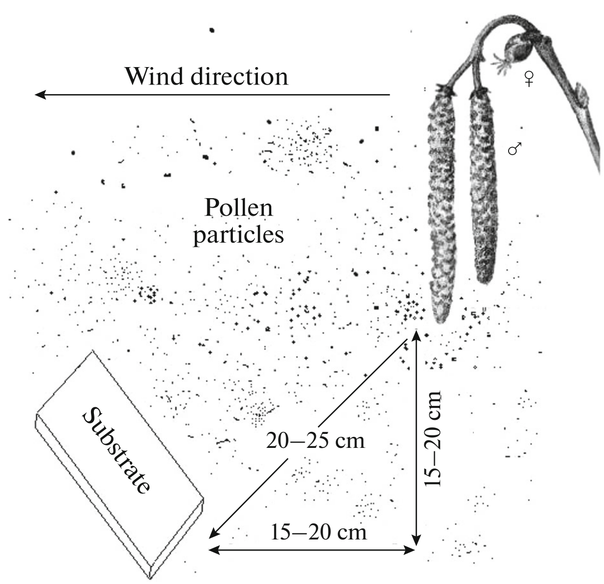

Fig. 1. Entrapment of *Corylus colurna* pollen at the time of its efflorescence from the anthers.

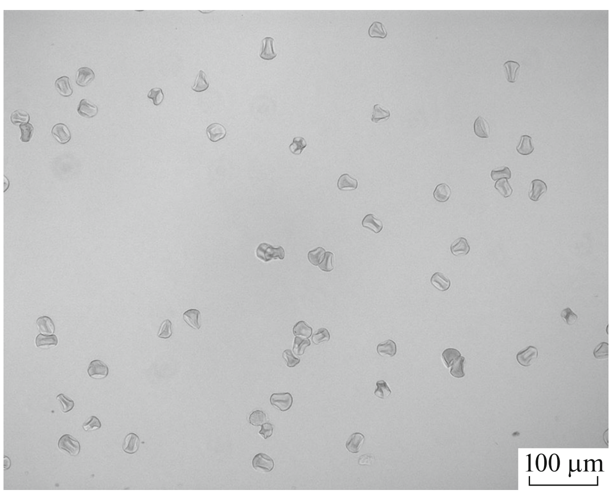

Fig. 2. Examples of PG clusters of *Agropyron cristatum*.

monitoring stations. Models of pollen transport created allowing for vegetation features, pollen production of plants, and processes of PP propagation seem to be more promising for forecasting the pollen content in the atmosphere.

The efficiency of the wind blowing PPs off the anther surface, the time of their stay in the air, distance of transport, and efficiency of capture by collecting surfaces depend on the sedimentation rate.

The PGs of anemophilous plants entering the atmosphere dry out and change their size, shape, and density of the protoplast [9, 10]. In the cytoplasm of a PG vegetative cell, large air spaces can appear [11]. The PG sedimentation rate is most strongly affected by the formation of clusters of two and more PGs [12], the process which is still poorly studied.

This work continues the studies [13–15] of the PA cluster composition during atmospheric pollen emission from 29 kinds of species of plants growing in the Central Siberian Botanical Garden (CSBG), Siberian Branch, Russian Academy of Sciences.

MATERIALS AND METHODS

All experiments were carried out in field conditions. We studied the cluster composition of PA entering the atmosphere from plants growing outdoors, both in natural populations and in collection areas of the CSBG. PPs were blown by wind from plant inflorescences to substrates covered with glycerin gelatin with addition of Coomassie blue (Fig. 1). The gust speed was measured by an anemometer and was 0.3–2.0 m/s. The substrates were arranged in the direction of the wind. The distance from the substrates was 20–25 cm, which made it possible to trap a sufficient amount of PPs and to avoid the contact of the substrates with inflorescences. Pollen of each species was sampled five times with intervals of several minutes. The exposure of the substrates lasted 1–2 s. Temperature and relative air humidity were simultaneously measured with a Center 311 device. The pollen particles (individual PGs and their clusters) were counted on ten transects at 10–40-fold magnification of the MBI-11U42 microscope lens.

Features of the PG morphology of anemophilous plants (the pollen is dry, with a thin and smooth outer shell (exine)) impede their coalescence; however, it is not clear how efficiently they interfere with cluster formation. It is supposed that individual PGs enter the atmosphere during the emission from anthers and the observed clusters are formed in the process of sedimentation directly on the microscope slide (Figs. 2 and 3).

The number of clusters of two and more PGs that would be formed on a substrate was estimated under the following assumptions: (i) sedimentation of PGs onto a substrate does not depend on sedimentation of other PGs and (ii) PGs are arranged in a layer in a forming cluster. With an increase in the number of PGs in clusters, their number on a substrate should decrease. If the mathematical expectation of the number of clusters consisting of an arbitrary number of PGs is less than unity, then similar (and larger) PPs are not formed on a substrate at a given number of PGs per unit area.

A cluster is formed if the distance between the geometric centers of PGs does not exceed two radii. Thus, the mathematical expectation of the number of clusters including two or more PGs ($N_{\geq 2}$) can be represented in the form

$$N_{\geq 2} = 4pN_{\geq 1}, \tag{1}$$

where $N_{\geq 1}$ is the number of PGs in clusters of one and more PGs (in fact, the total number of PGs settled on the substrates);

$$p = S_{pg}N_{\geq 1}/S_{T} \quad (2)$$

is the fraction of the substrate surface occupied by PGs (see Fig. 1), S_{pg} is the average area of the PG projection, and S_T is the area of the substrate examined.

To estimate the area of individual PGs, photographs of ~200 PGs of all examined plant species were taken in the Common Use Center for Microscopic Analysis of Biological Objects of the Siberian Branch, Russian Academy of Sciences. The areas of PG projections in the photographs were determined from image processing with the MapInfo Professional software.

The mathematical expectation of the number of clusters of two PGs can be estimated by the relationship

$$N_2 = N_{\geq 2} - N_{\geq 3}, \quad (3)$$

where $N_{\geq 3}$ is the mathematical expectation of the number of clusters consisting of at least three PGs,

$$N_{\geq 3} = 7pN_{\geq 2}. \quad (4)$$

In the general case, mathematical expectations of the number of clusters of j and more PGs can be represented in the form

$$N_{\geq j} = (3(j-1)+4)\,pN_{\geq (j-1)}, \quad (5)$$

$$N_j = N_{\geq j} - N_{\geq j+1}. \quad (6)$$

The mathematical expectation of the number of individual PGs

$$N_1 = N_{\geq 1} - 2N_2 - 3N_3 - \ldots - jN_j. \quad (7)$$

In the process of PG settling on a substrate, two alternative variants are possible: PGs appear on the substrate either individually or as components of clusters of two or more PGs. Therefore, the problem can be reduced to comparison of fractions of individual PGs of the total number of PGs settled on the substrates, i.e., to estimation of the significance of differences between fractions or percentages of the feature characterized by an alternative distribution. For this purpose, the Fisher criterion F with the φ-transformation (Fisher angular transformation) was used. It is intended for comparison of two samples according to the frequency of occurrence of an index a researcher is interested in:

$$F = \frac{(\varphi_1 - \varphi_2)^2 N_{a\geq 1} N_{b\geq 1}}{N_{a\geq 1} N_{b\geq 1}} \sim F_{(a,\, df_1\, df_2)}, \quad (8)$$

where φ_1 and φ_2 are the transformed fractions and $N_{a\geq 1}$, $N_{b\geq 1}$ are the sample volumes (the total numbers of PGs on the substrates). The value obtained was compared with the tabular one at a given level of significance and the number of the degrees of freedom $df_1 = 1$; $df_2 = N_{a\geq 1} + N_{b\geq 2} - 2$.

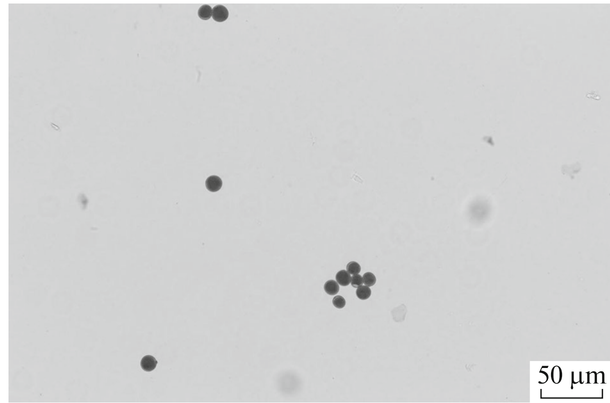

Fig. 3. Density of the PG sediment of *Poa pratensis* on a substrate.

When the sample volumes number in hundreds and thousands, $F = 3.8$ at the significance level $\alpha = 0.05$, 6.6 at $\alpha = 0.01$, and 10.8 at $\alpha = 0.001$. If the calculated values of F exceed the abovementioned values, the null hypothesis at a given level of significance should be rejected.

RESULTS

Emission of pollen entering the atmosphere directly from anthers of 29 plant species growing in the CSBG, including 26 anemophilous plant species: seven species of woody plants, 14 species of grasses, and five species of weeds (herbs) have been studied. The data about the presence of clusters in the pollen of the plants under study are shown in Table 1. Clusters were revealed in pollen samples of all plants under study. The percentage of clusters of two and more PGs reached 71% of the total number of PPs (*Alnus hirsuta*); the PG percentage in their composition was 93.7% (*Populus alba*) of the total number of trapped PGs.

The results of the statistical analysis of plant samples are presented in Table 2.

The Fisher criterion varied from 16.9 (*Cyperus papyrus*) to 208 (*Populus alba*), which is obviously higher than its value even at a significance level of 0.001 (10.8). The null hypothesis about formation of clusters from individual PGs on the substrates should be rejected.

Pollen of entomophilous plants significantly differed in its cluster composition ($\alpha = 0.001$) from the PA produced by anemophilous plants. For example, comparison of percentages of individual PGs produced by *Oxalis acetosella* and *Chosenia arbutifolia* yields $F = 42.3$.

Table 1. Presence of clusters in the plant pollen entering the atmosphere

Specific name	Observation date	*t*, °C	RH, %	Number		Percentage of clusters of two and more PGs, %	PG percentage in clusters of two and more PGs, %
				PPs	PGs		
Anemophilous plants							
Pinus mugo	June 13, 2020	27.0	54.0	3195	5762	36.0	64.5
Carex cespitosa	June 1, 2021	34.2	32.7	2072	2500	12.7	27.7
Agropyron cristatum	June 23, 2020	26.8	52.7	4293	5143	13.4	27.7
Agrostis capillaris	June 23, 2020	32.0	43.3	1351	2019	24.6	49.5
Cyperus papyrus	June 3, 2021	22.4	62.8	494	607	12.3	28.7
Festuca rubra	June 8, 2020	29.7	95.6	921	1259	21.0	42.2
Sanguisorba officinalis	July 26, 2017	28.8	42.6	1637	1963	14.7	28.9
Corylus colurna	May 3, 2021	14.2	30.7	4469	5356	13.5	27.8
Festuca ovina	June 2, 2020	22.4	61.9	1352	1593	12.1	25.4
Poa pratensis	June 8, 2020	26.6	49.7	396	566	16.4	41.5
Helictotrichon sempervirens	June 1, 2021	35.9	20.8	422	685	25.4	54.0
Amaranthus caudatus	Sept. 9, 2019	24.4	50.7	822	1572	35.4	66.2
Carex leporina	June 1, 2021	31.7	41.8	670	914	19.1	40.7
Koeleria glauca	June 8, 2020	28.8	51.3	1604	2206	18.6	40.8
Calamagrostis acutiflora	Sept. 9, 2019	23.0	38.5	676	909	19.1	39.8
Fraxinus pennsylvanica	May 15, 2021	23.6	19.7	4917	6195	15.7	33.1
Triticum aestivum	July 26, 2019	24.2	72.7	514	730	18.5	42.6
Triticum durum	July 26, 2019	29.6	65.0	428	659	26.9	52.5
Microbiota decussata	Apr. 30, 2019	26.7	24.5	1539	2212	22.3	45.9
Festuca glauca	June 8, 2020	25.7	65.4	1145	1645	17.6	42.6
Briza media	June 23, 2020	37.0	46.9	584	1175	33.7	67.1
Alnus hirsuta	Apr. 26, 2019	12.2	47.8	3805	7877	71.0	86.0
Chosenia arbutifolia	May 8, 2019	22.2	37.4	380	462	15.5	30.5
Populus alba	Apr. 26, 2017	17.6	53.2	2609	18321	55.4	93.7
Pennisetum alopecuroides	Sept. 9, 2019	28.5	80.1	3394	4693	19.4	41.7
Avenella flexuosa	June 8, 2020	26.6	51.7	3499	4722	16.9	38.4
Entomophilous plants for which wind pollination is possible							
Oxalis acetosella	Aug. 9, 2018	39.5	54.0	487	1347	38.8	77.9
Tilia amurensis	July 13, 2019	27.7	39.6	1669	2489	25.3	49.9
Tilia platyphyllos	July 10, 2019	26.9	47.7	496	1240	40.5	76.2

Table 2. Number (n_j) and mathematical expectation (N_j) of PG clusters

Specific name	$N_{\geq 1}$	p, %	Parameter	Number j of PGs in the cluster composition												F
				1	2	3	4	5	6	7	8	9	≥10	≥20	≥100	
Anemophilous plants																
Pinus mugo	5762	0.98	n_j	2046	566	268	140	73	37	19	8	4	28	6		69.0
			N_j	5293	211	14	1									
Carex cespitosa	2500	0.13	n_j	1808	185	38	21	9	5	1	3	2				31.8
			N_j	2416	13											
Agropyron cristatum	5143	0.37	n_j	3716	447	67	26	21	6	2	3	1	4			38.7
			N_j	4989	74	2										
Agrostis capillaris	2019	0.15	n_j	1019	178	68	44	16	14	3	4	3	2			39.2
			N_j	1995	12											
Cyperus papyrus	607	0.09	n_j	433	45	5	2	3	2	1	1	1	1			16.9
			N_j	603	2											
Festuca rubra	1259	0.07	n_j	728	135	34	4	4	6	3	2	0	5			31.5
			N_j	1251	4											
Sanguisorba officinalis	1963	0.06	n_j	1396	192	35	7	4	0	0	0	0	3			53.4
			N_j	1955	4											
Corylus colurna	5356	0.14	n_j	3865	430	120	31	11	8	2	0	0	1	1		46.5
			N_j	5296	30											
Festuca ovina	1593	0.10	n_j	1188	135	12	5	3	5	2	0	0	2			24.9
			N_j	1581	6											
Poa pratensis	566	0.02	n_j	331	25	17	8	5	4	2	0	1	3			21.6
			N_j	564	1											
Helictotrichon sempervirens	685	0.05	n_j	315	55	17	11	6	7	4	1	3	3			28.6
			N_j	683	1											
Amaranthus caudatus	1572	0.04	n_j	531	125	73	37	13	11	9	9	2	10	2		50.1
			N_j	1568	2											
Carex leporina	914	0.05	n_j	542	58	46	14	6	2	0	0	0	2			26.7
			N_j	910	2											
Koeleria glauca	2206	0.12	n_j	1305	153	83	28	12	7	4	7	2	3			39.7
			N_j	2186	10											
Calamagrostis acutiflora	909	0.07	n_j	547	75	26	16	6	4	0	2					26.3
			N_j	905	2											
Fraxinus pennsylvanica	6307	0.18	n_j	4253	505	145	52	30	8	5	4	4	10			54.5
			N_j	6214	46	1										
Triticum aestivum	730	0.08	n_j	419	51	18	6	7	5	2	3	2	1			23.7
			N_j	726	2											
Triticum durum	659	0.07	n_j	313	64	21	17	4	4	2	0	1	2			27.1
			N_j	655	2											

Table 2. (Contd.)

Specific name	$N_{\geq 1}$	p, %	Parameter	Number j of PGs in the cluster composition												F
				1	2	3	4	5	6	7	8	9	≥10	≥20	≥100	
Microbiota decussata	2212	0.18	n_j	1196	213	71	23	14	10	1	1	0	6	4		41.3
			N_j	2179	16											
Festuca glauca	1645	0.11	n_j	944	130	18	14	7	8	7	3	5	5	4		35.5
			N_j	1631	7											
Briza media	1175	0.10	n_j	387	70	38	22	23	17	6	10	3	8			42.5
			N_j	1167	4											
Alnus hirsuta	3897	0.28	n_j	1103	322	128	86	46	30	18	25	14	20	12		75.6
			N_j	3807	43	1										
Chosenia arbutifolia	462	0.01	n_j	321	37	21	1									17.8
			N_j	462												
Populus alba	18 321	0.63	n_j	1163	318	194	93	82	60	43	47	29	270	248	62	208.0
			N_j	17373	443	19	1									
Pennisetum alopecuroides	4693	0.09	n_j	2734	403	118	64	21	21	10	6	6	8	3		54.2
			N_j	4659	16											
Avenella flexuosa	4722	0.10	n_j	2908	344	100	58	26	26	16	10	4	6	1		56.4
			N_j	4685	12											
Entomophilous plants for which wind pollination is possible																
Oxalis acetosella	1347	0.03	n_j	298	90	33	18	10	8	2	5	4	11	5	3	53.3
			N_j	1343	2											
Tilia amurensis	2489	0.11	n_j	1246	227	103	45	27	6	9	2	0	3	1		48.7
			N_j	2467	11											
Tilia platyphyllos	1240	0.06	n_j	295	73	42	22	13	7	4	11	8	13	8		49.4
			N_j	1234	3											

CONCLUSIONS

Results of studying the pollen entering the atmosphere for ~1/12 of the total number (417) of species represented in the flora of Akademgorodok, Novosibirsk, corroborate preliminary conclusions about the character of the pollen emission [9–11]. The anemophilous plant pollen entering the atmosphere is not monodisperse. In addition to individual PGs, clusters containing two and more PGs enter the atmosphere. The percentage of such clusters of the total number of forming particles varies over a wide range and can be markedly different in different plant species.

The following conclusions can be drawn from the work:

(1) Morphological features of the structure of pollen grains of anemophilous plants do not prevent the formation of clusters in the process of pollen emission into the atmosphere.

(2) Anemophilous plant pollen entering the atmosphere is not monodisperse but represented both by individual PGs and by clusters of two and more PGs.

(3) The fraction of clusters of the total number of particles formed and the percentage of PGs in their composition vary over a wide range and can reach 71 and 93%, respectively.

(4) Anemophilous plant pollen entering the atmosphere significantly differs from pollen of entomophilous plants in its cluster composition.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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