MORPHOLOGICAL CHARACTERISTICS OF POLLEN *BETULA VERRUCOSA* EHRH. (SYN. *B. PENDULA*) DEPENDING ON HABITAT

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Summary. As a result of carrying out by means of scanning electronic microscopy of the comparative analysis of morphological traits of two samples of pollen Betula verrucosa Ehrh. from different habitats, it is revealed reliable distinctions between length of a polar axis, equatorial diameter and the area of apoporial field. The length of the polar axis of the samples of dry pollen grains from Kiev defined from 16.49 to 22.26 μm and from the samples of dry pollen grains from Ivankov from 16.88 to 22.51 μm. The length of the equatorial diameter from 18.95 to 26.96 μm and from 20.95 to 28.08 μm accordingly.

INTRODUCTION

For last century the ecological situation all over the world has appreciable worsened and, moreover, does not improve. Pollution of an atmosphere and hydrosphere anthropogenic industrial wastes, emissions of every possible types of transport, influence of the radiation factor on all alive, electromagnetic influence, genetically modified products, pathogenic bacteria and viruses – that in any way it is impossible to name favorable for a life not only the person, but also the animal and vegetative worlds. According to the World Health Organization, 40 % of diseases all over the world are caused by ecological factors of a physical, chemical and biological origin. In result the person is ill or his organism cannot resist even to insignificant negative influences any more, animals die out, plants disappear or change the properties [Kyoto protocol, 1998; Ilkyn, 1971; Yablokov, 2002; Yagodkina, 2009].

Allergic diseases are known to the people more than two thousand years, but only for last decades the problem of an allergy has accepted scale of a global medical-social problem. Now allergic diseases on the prevalence take the third place after cardiovascular and oncological, and in some ecologically adverse regions come out on top [Fedoskova, 2004]. The numerous epidemiological researches, spent for different regions of the world, convincingly show increase in prevalence of a pollen allergy both among adults and children. Pollinosis suffer to 39 % of the population of a planet [Shamgunova, 2010]. But pollen of plants in itself cannot be the direct reason of pollinosis [Shichzhen, 2010]. The reason is presence of allergens. One of the first places among wood plants which cause pollinosis, belongs to a silver birch (*Betula verrucosa* Ehrh.). The tree has a wide circulation: Europe, except for Iberian Peninsula, Northern Africa, Forward and the Central Asia, also introduced everywhere in a zone of a temperate climate. Its young leaves, not revealed buds, birch sap and even pollen [Mironenko, 2002] possess curative properties. During the period with 1997 for 2007 year it has been published 335 scientific works devoted to studying of birch pollen [Hilaire, 2007]. By researches it is shown, that buds of a *Betula verrucosa* Ehrh. keep the medical properties, growing in different, on a level of influence of negative factors of an environment, regions [Garkava et al, 2010]. But birch pollen on the contrary – changes properties under influence of factors of an environment. First of all the form and density of pollen grains change during their stay in an atmosphere [Hilaire, 2007]. Having got in an atmosphere, pollen settles on a surface after precipitation. Then pollen tubes start to sprout and burst, releasing tiny particles with allergens [El-Ghazaly et al., 1999]. Or water-soluble allergens can be washed up also from untouched settled pollen grains, and then to rise in air [Schappi, 1997]. Taking into account that a birch is wind pollinated tree and from one inflorescence is on the average allocated 10, 044000 pollen grains in the size 10-25 μm [Piotrowska, 2008; Blackmore, 2003]; its pollen contains about 40 proteins, 6 from which possess properties of allergens [Kalinina, 2007], then on the one hand birch pollen has the reason to be allergic. And on the other hand, pollen of a birch is not «full» allergen. The main allergen of a birch, so-called Bet v 1, glycoprotein – a protein-polysaccharide complex which acts as signal substance for germination of pollen on stigma a pestle and probably renders protection

against pathogenic microorganisms [Emilson et. al 1996, El-Ghazaly et. al, 1999]. And only getting on mucous membranes of a nose, an eye of the person birch pollen turns on strong allergen as the human body perceives it as object carrying alien.

In references the morphological description of pollen *Betula verrucosa* Ehrh. is resulted [Erdtman, 1943; Kupriyanova, 1972; Blackmore, 2003]. Pollen grains are monad; pollen class: small (10-25 μm), isopolar; shape: spheroidal; equatorial outline: circular. P/E ratio: suboblate to oblate. Aperture type: 3-porate, annulate, operculate, oncus. Ectoaperture – pore more or less circular to slightly elliptic, margins smooth and distinct, with a distinct aspis. Nexine and sexine separated to form a distinct, dome-shaped vestibulum. Endoaperture – pore of similar diameter to the ectopore. Aspides distinctly protruding. Exine: Thin – 0.8-1.4 μm. Sexine up to three times as thick as nexine. Sexine 1 of short columellae which are not usually visible under LM. Sexine 2 a the tectum, twice as thick as sexine 1. Sexine 3 of scabrae on ridges (SEM, or just visible with oil immersion). Ornamentation: granulate, rugulate. In LM psilate, scabrate in SEM. Scabrae short and conical, on short, irregularly arranged micro-rugulate ridges. The ridges may be more or less distinctly defined. Columellae circular, often in irregular, short rows which correspond with the ridges. Tectum: eutectate; cell no.: 2-celled. Additional info: LM: psilate, unknown; pollen wall: columellate; footlayer continuous; endexine absent; intine standard; chemical components: primexine matrix absent, pollenkitt absent, tryphine absent, pollen coating vesicles absent, lipids absent, polysaccharide vesicles absent, starch present; Ubisch bodies: present. Dry pollen: shape: irregular; outline: irregular; infoldings: irregularly infolded; annotations: tectum very mighty. Dry pollen infoldings irregular infolded but only interapertural areas sunken. Color of grains are yellowish. As if to the size of pollen grains of a birch in references cited the diverse and incomplete data: 24.3 μ [Jentys-Szaferowa, 1928]; 21.8 μ [Welten, 1944]; 22.1 μ [Eneroth, 1951]; 25.1 μ [Koperowa and Srodon, 1965]; 20-22 μm [Sofiev, 2007]; 21.34 μm x 18.17 μm [Piotrowska, 2008].

With the purpose of revealing changes in pollen of a birch under influence of ecological factors it has been carried out a comparative estimation of morphological traits of pollen grains *Betula verrucosa* Ehrh. from different places of growth.

MATERIAL AND METHOD

Pollen of a *Betula verrucosa* Ehrh. has been prepared prior to the beginning of anthesis during the period from April, 18 till April, 24, 2011 in Kiev region territory. For research 2 samples of birch pollen have been selected from the Kiev region, namely: 1. Kiev (pollen is collected from the birches growing in a park zone); 2. utc. Ivankov the Kiev region, concerning to III Chernobyl zone according to definition of a belonging of territory as such the Ministry of Ukraine on questions of extreme situations and on affairs of protection of the population from consequences of Chernobyl accident [Ministerstvo…, 2008] also is on distance of 80 km to a southwest from Kiev (pollen is collected from the birches growing near highways and apartment houses). These regions have been chosen in view of their different latitude of a site, a different radiating background and different anthropogenic loading. Also differences will consist in date of preparation of catkins of a birch and as result of a degree of their maturity (fig. 1). Maturity defined on color of birch catkins, and also on a degree of blooming of leaves: more mature catkins have greenish-yellow, then light yellow painting and was much longer (70 mm, a maximum of 100 mm agree Piotrowska, 2008).

For researches used preliminary dried up pollen. Morphological traits of pollen grains were measured. Scanning electronic microscope ZEISS EVO LS 15 was used for research of morphological traits. A thin layer of pollen grains were placed on an objective little table of a microscope on special sticky basis. Morphological traits were measured and estimated for 140 pollen grains (i.e. on 20 for each sample). For measurements used license program AxioVs40 V 4.8.2.0 (Carl Zeiss, Jena, Germany). Polar axis (P – the straight line between the distal and proximal poles of a pollen grain), length equatorial axis (E – pollen width, the distance between the poles in equatorial part of pollen), length of an apoporial field edge of pollen grains, angel

arrangements of apertures to a contour of a pollen grain, internal diameter of apertures were measured.

Figure 1 Maturity of catkins of *Betula verrucosa* Ehrh.
(Photo: T. Shevtsova 2011)

Great volume of sample suffices at measurement of pollen grains for each kind has been accepted equal 60, paid off on sample with the greatest factor of a variation at confidential probability $q=0.99$ and relative discrepancy ε no more than 5 % [Urbach, 1963]. An estimation of reliability of distinctions between average values and dispersions samples carried out accordingly by t-test and f-test, at 3 % a significance value. At the analysis of the data also used nested ANOVA. Results were processed in system STATISTICA 6.1.

RESULTS AND DISCUSSIONS

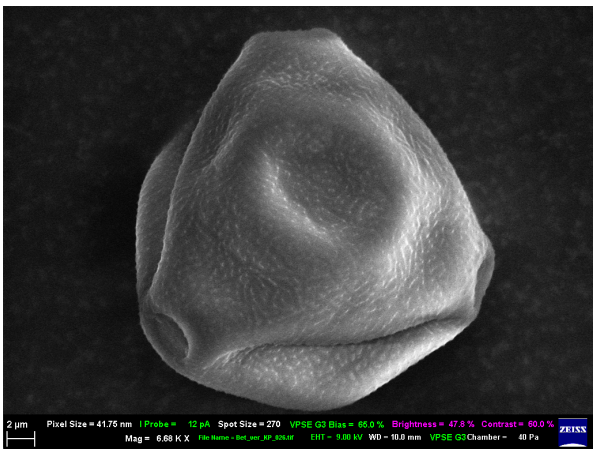
The above-stated parameters of pollen grains *Betula verrucosa* Ehrh. have been measured. Results are shown in table 1. In figure 2 it is submitted photos of birch pollen.

Table 1 Variability of morphological traits of pollen grains of *Betula verrucosa* Ehrh., (µm)

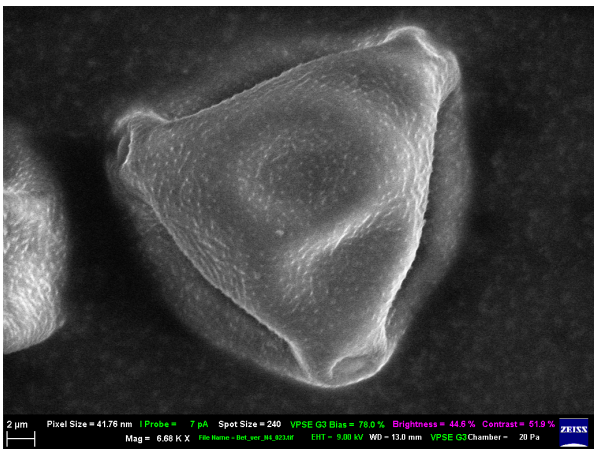
Sample	*n*	*min*	*max*	$\bar{x}$	*σ*	*V %*	*t*	*Paldat (2003)*	*Polleninfo*	*Покровская И.М. (1950)*
Length of the polar axis (P)										
Kiev	*60*	*16.49*	*22.26*	*18.87*	*1.34*	*7.08*	*2.31*$^{+}$	*17.0-(19.5)-24.0*	*21.0-(22.6)-25.0*	*No data*
Ivankov	*60*	*16.88*	*22.51*	*19.48*	*1.56*	*8.02*				
Length of the equatorial diameter (E)										
Kiev	*60*	*18.95*	*26.96*	*23.26*	*1.92*	*8.25*	*2.86*$^{++}$	*22.0-(24.5)-28.0*	*22.0-(24.0)-25.0*	*15.0-(19.6)-21.0*
Ivankov	*60*	*20.95*	*28.08*	*23.98*	*1.66*	*6.94*				
Length of an apoporial field edge										
Kiev	*60*	*22.62*	*31.05*	*26.42*	*1.92*	*7.28*	*2.22*$^{+}$	*No data*	*No data*	*No data*
Ivankov	*60*	*22.58*	*32.71*	*27.40*	*1.83*	*6.68*				
Angel arrangements of apertures										
Kiev	*60*	*83.80*	*125.85*	*105.40*	*7.61*	*7.22*	*3.23*$^{++}$	*No data*	*No data*	*No data*
Ivankov	*60*	*86.55*	*115.23*	*101.13*	*6.86*	*6.78*				
Length of the internal diameter of apertures										
Kiev	*60*	*2.06*	*4.27*	*3.26*	*0.44*	*13.37*	*0.51*	*No data*	*No data*	*No data*
Ivankov	*60*	*2.53*	*3.89*	*3.25*	*0.33*	*10.19*				

n – number of measurements; min – the minimal value; max – the maximal value; x – arithmetic mean of sample; σ – standard deviation; V – coefficient of variation (%); t – t-test
*$P < 0.05$; **$P < 0.01$.

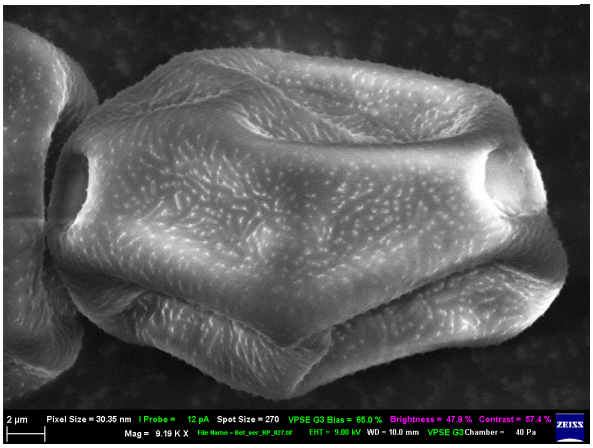
Apparently from table 1 the most part of traits, namely, the length of a polar axis both equatorial diameter, the area of apoporial field and an angel of an arrangement of apertures of pollen grains, reliable differ among themselves with a significance value 0.05. These results are well illustrated on Box & Whisker Plots (fig. 3).



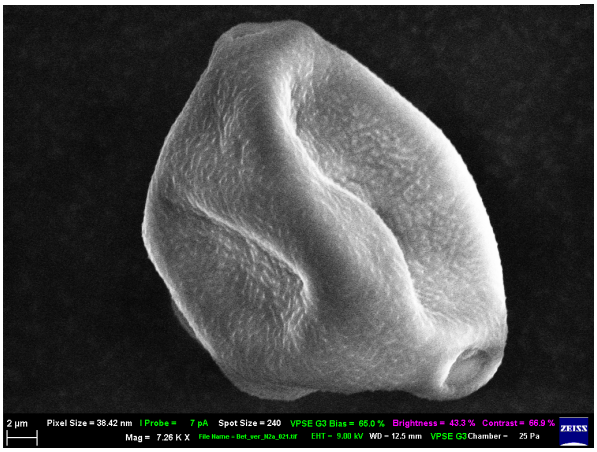
Polar view, Kiev



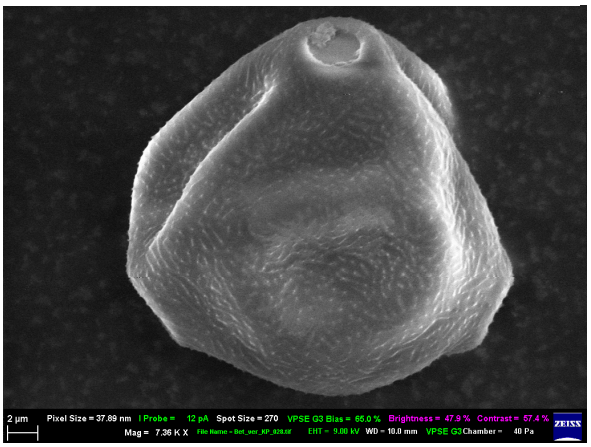
Polar view, Ivankov



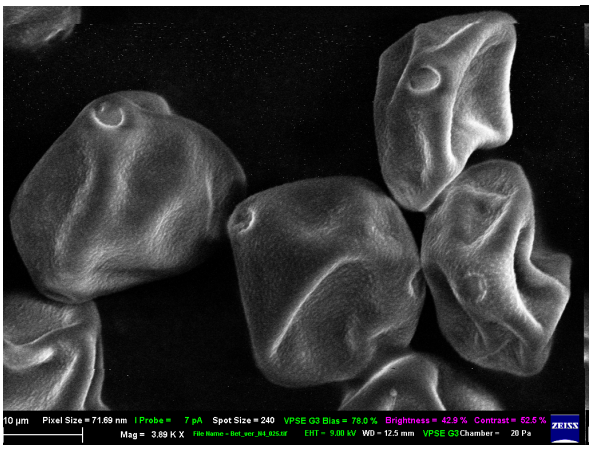
Equatorial view, Kiev



Equatorial view, Ivankov



Polar view, Kiev



Polar view, Ivankov

Figure 2 Comparison of morphological traits samples of *Betula verrucosa* Ehrh. pollen grains:
(Photo: R. Ostrovsky, T. Shevtsova 2011)

The received data do not contradict the literary data. The analysis of the received data has shown, that length of a polar axis both equatorial diameter and the area of apoporial field of pollen grains *Betula verrucosa* Ehrh. which have been collected in Ivankov, is reliable more, than the pollen collected in Kiev. A place of gathering utc. Ivankov there is to the north, rather than Kiev on 29′ N. However gathering of catkins in Ivankov passed for 6 days later, than in Kiev, that for the general period of active flowering a birch (5-10 days) is a significant difference in date of gathering. Besides the level of anthropogenic loading in Ivankov is much lower, than in Kiev, that also matters. As if to radiating influence its action on morphology of pollen of a birch is not revealed by us.

The received results give the basis to speak what to study a plant, in our case pollen, at a level of a species is insufficiently. It is necessary to do it at least on a population of species. The nature has taken care of a variety of all alive. This variety is shown and in geographical conditions of growth of a plant, and in various climatic factors, and in a degree of influence of the human factor. Under the theory of chaos there are no two identical objects in the universe. Therefore there are no two identical pollen grains from the same stamen.

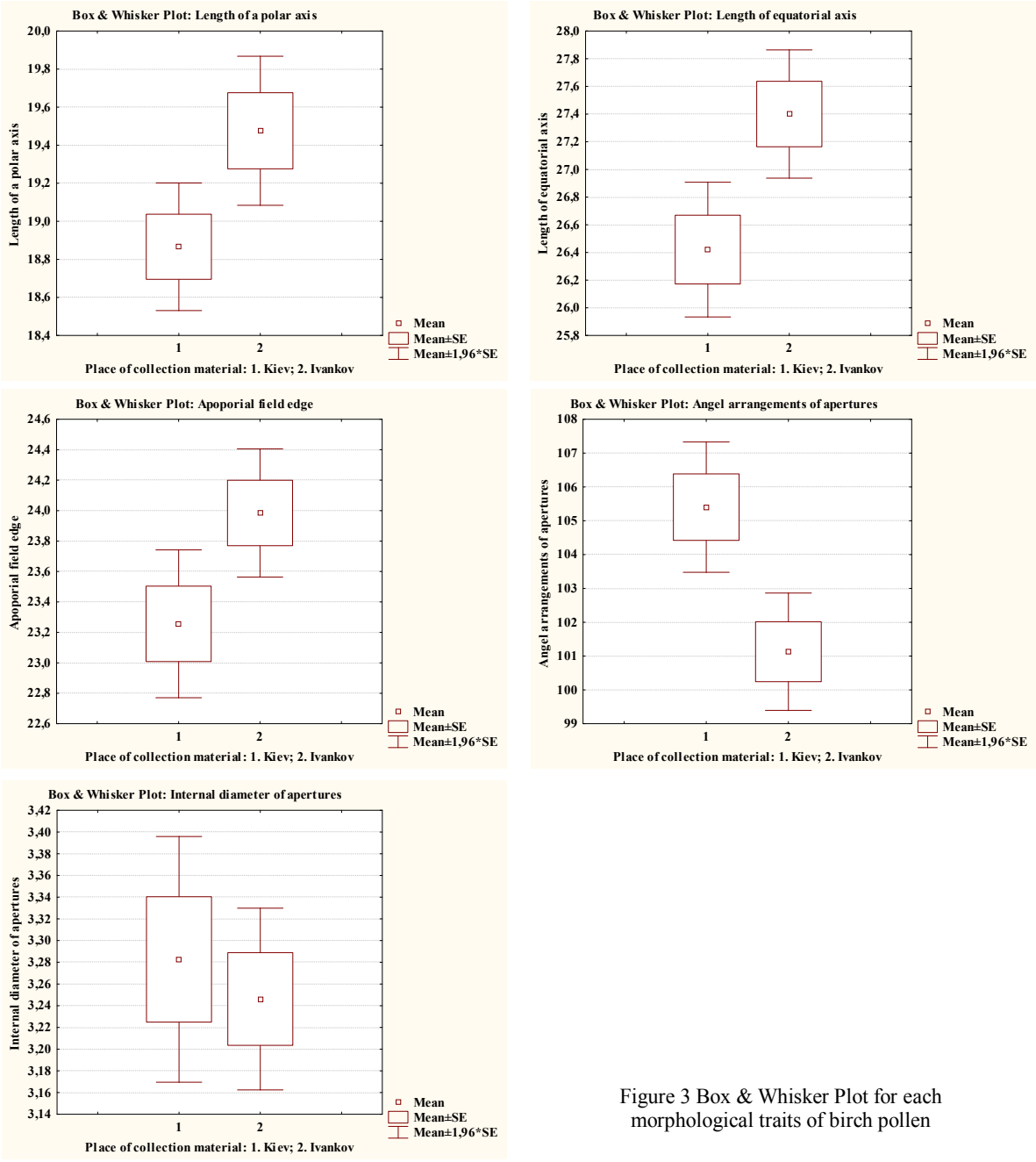


Figure 3 Box & Whisker Plot for each morphological traits of birch pollen

CONCLUSIONS

Thus, morphometrical parameters of pollen grains *Betula verrucosa* Ehrh. from two places of growth have been investigated by us. Work has been focused on the comparative analysis of morphological traits and characteristics of conditions of research objects growth. It is revealed, that a main role on influence on morphology of pollen and change of its properties two factors have played: anthropogenic loading of a place of growth of objects of research and date of gathering of a material. Both of these factors have come to light more favorable in Ivankov. Reliable distinctions between values of morphological traits are confirmed statistically.

In the subsequent researches it is necessary to study the same characteristics for pollen of a birch from other habitats. Revealing of reliable distinctions of one kind in territory of one region or the neighboring regions, will enable to make more a substantiated conclusion about variability of birch pollen under influence of adverse factors.

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