

IDENTIFICATION OF OUTDOOR POLLEN - RESULTS FROM A POLLEN TRAPPING EXPERIMENT

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Abstract

Many allergenic plants are wind-pollinated and produce large amount of pollen that has the ability to float in the air. An efficient preventive treatment for pollen allergy is the avoidance of exposure. This can be done if there is a detection of the regional atmospheric concentrations for various pollen types. The present study shows the results of an outdoor pollen trapping experiment in the vicinity of an area rich in vegetation, the Carol Park in Bucharest, during a week in May 2018. Pollen analysis was done using stained microscope slides at a magnification of 400×. Microscopic images of all the pollen grains on the slides were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens). Eight pollen types were identified, these were: inaperturate; monoporate; triporate; pollen with 4 pores; periporate; tricolpate; tricolporate and saccate pollen.

Key words: Carol Park (Bucharest), light microscope (LM), microphotographs, outdoor pollen.

INTRODUCTION

Pollen cytoplasm contains some allergenic proteins that normally have a role in fertilization in plants, however, since pollen size is small (microscopical) and because these allergenic proteins are water-soluble and can be released, they can reach other targets too (humans, animals) causing allergy. Pollen allergy (pollinosis) is one of the most common respiratory allergy in Romania. The incidence of this type of allergy has been increasing in Europe in the past decades (D'Amato, 2007).

Many allergenic plants are wind-pollinated and produce large amount of pollen that has the ability to float in the air (Ianovici, 2007). An efficient preventive treatment for pollen allergy is the avoidance of exposure. This can be done if there is a detection of the regional atmospheric concentrations for various pollen types. Several European scale projects aiming to develop such a system of forecasting hourly pollen concentrations all over Europe are being carried out (European Aerobiology Society, 2020), also there are specific training courses and meetings for aerobiologists (Berger, 2020a). A standard methodology is applied for the measurements of the pollen content in the air - pollen traps are used and a determination and counting of pollen grains using light microscopy (Berger, 2020b;

Galan et al., 2014). Identification of pollen types is based on the knowledge of the pollen morphology and description for the pollen types usually found in air samples and is carried out at magnification 400× (Bucher & Kofler, 2020). At present, in Romania, there is a need to develop a network of aerobiological monitoring laboratories. Therefore, following previous studies (Enache et al., 2019), an attempt to identify different pollen types from the air in late spring, using a pollen trapping experiment carried out in the vicinity of an area rich in vegetation, was considered relevant.

MATERIALS AND METHODS

Carol Park is one of the oldest parks in Bucharest (dates from 1906) and is well known for several monuments of historical value as well as the beautiful panoramic views and vegetation, which includes numerous trees (Figure 1). To analyze pollen types found in the air, 10 microscope slides with a sticky surface were kept outdoors on a 1st floor balcony of a house situated next to the park for a week in May 2018 (6-11 May), then they were brought to the Laboratory of Biology of the Faculty of Biotechnologies of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.



Figure 1. View from Carol Park in winter (the Mausoleum monument can be seen on the back left)

Slides were stained with toluidine blue for better contrast and a coverslip was added (Figure 2). An optical microscope Micros Austria was used (ob. 40×) and the microscopic images of all the pollen grains on the slides were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens). The measurements were done with an ocular micrometer and the calibration ratio was 2.5 μm.



Figure 2. Slides used in the present study

Comparisons of the images were made with pollen descriptions found in the literature (Tarnavski et al., 1981; 1987; 1990; Șerbănescu-Jitariu et al., 1994) or on various Internet sites (Pollen-Wiki-Der digitale Pollenatlas, Stebler, 2020a; the PalDat-Palynological Database or the Pollen Atlas of Bucher and Kofler, 2020), as well as with our collection of allergenic pollen images (Enache et al., 2019). The classification of pollen according to size is from Stebler (2019b).

RESULTS AND DISCUSSIONS

The list of main plant genera/species of relevance for aerobiological studies found in Romania (Berghi, 2012) includes species that have pollen that can be classified in one of the following 8 pollen types:

- inaperturate: Cupresaceae - *Juniperus* sp. (junipers); Salicaceae - *Populus* sp. (poplar);
- monoporate: Poaceae - wild grasses and cultivated grasses (including ornamental grasses);
- diporate: Moraceae - *Morus* sp. (mulberry);
- triporate: Urticaceae - *Urtica* sp. (nettle), *Parietaria officinalis* (common pellitory); Betulaceae - *Corylus* sp. (hazel), *Betula* sp. (birch);
- 4 to 6 pores: Betulaceae - *Carpinus* sp. (hornbeam), *Alnus* sp. (alder); Ulmaceae - *Ulmus* sp. (elm);
- periporate: Plantaginaceae - *Plantago lanceolata* (plantain); Amaranthaceae - *Amaranthus* sp.; Juglandaceae - *Juglans* sp. (walnut);
- tricolpate: Aceraceae - *Acer* sp. (maple); Fagaceae - *Quercus* sp. (oak); Platanaceae - *Platanus* sp. (plane trees); Oleaceae - *Fraxinus* sp. (ash); Asteraceae - *Ambrosia elatior*, *A. trifida* (ragweeds), *Xanthium strumarium*, *X. commune* (cockleburs);
- tricolporate: Fagaceae - *Castanea* sp. (chestnut), *Fagus* sp. (beech); Polygonaceae - *Rumex* sp. (dock, sorrels); Asteraceae - *Artemisia vulgaris* (mugwort); Tiliaceae - *Tilia* sp. (lime tree/linden); Salicaceae - *Salix* sp. (willow); Oleaceae - *Ligustrum* sp. (privet).

Differences between plant genera/species in one category can be determined based on pollen descriptions in each case (Bucher and Kofler, 2020).

For the microscope slides that were analysed a total of 456 microphotographs were obtained and 8 pollen types were identified.

Only one granule was inaperturate; while several pollen grains were present in each of the other 7 categories. Bisaccate medium to very large pollen grains (Pinaceae) were over-represented on these slides (Figure 3).

Small or medium size monoporate pollen grains were also seen, due to their characteristic shape and annulus present around the pore, they can be identified as Poaceae (Figure 4).



Figure 3. Large bissacate pollen grain (~ 80 μm)

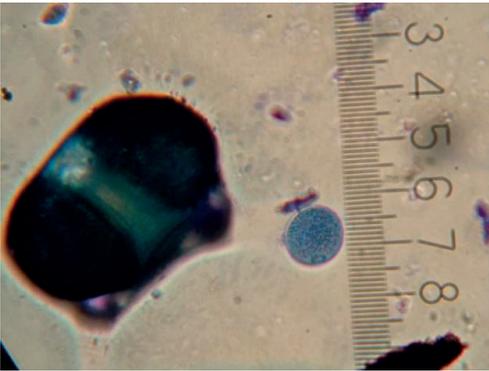


Figure 4. Medium size monoporate pollen grain (~ 30 μm) with annulus next to very large bissacate pollen grain (~ 115 μm)

Hazel and birch have similar shapes and have both 3 protruding pores (Figure 5), however these are larger in hazel therefore in polar view birch looks more circular (Figure 6).



Figure 5. Triporate pollen grain with protruding pores (~ 22,5 μm) - semi-lateral view

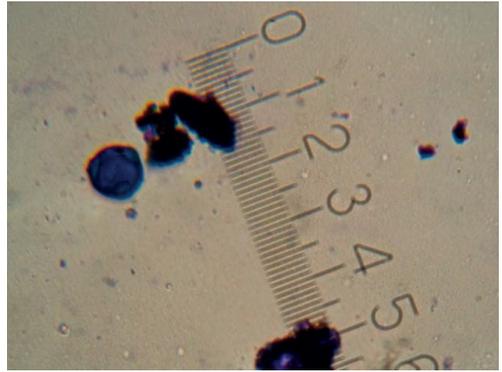


Figure 6. Triporate pollen grain (~ 25 μm) - apical view

Four or five pores have hornbeam and alder, both are circular in polar view (Figure 7), but alder has distinctive protruding pores. Also there is a size difference between them: alder pollen is small, while hornbeam is medium size.

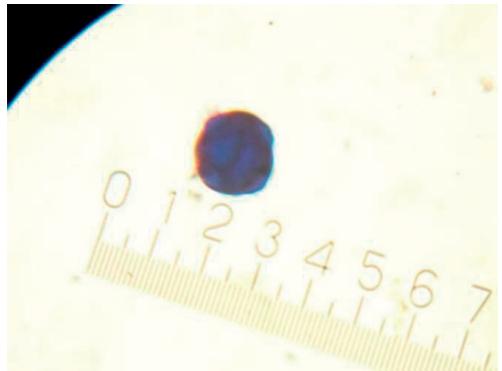


Figure 7. Pollen grain with 4 pores (~ 37.5 μm) - apical view

Two types of round, medium size periporate pollen grains were also seen on the slides in the present study (Figures 8-9), however there are details that differentiate them - one is operculate (possible *Plantago lanceolata*), while the second type has pores with a lenticular thickening around them (*Jugans* pollen).

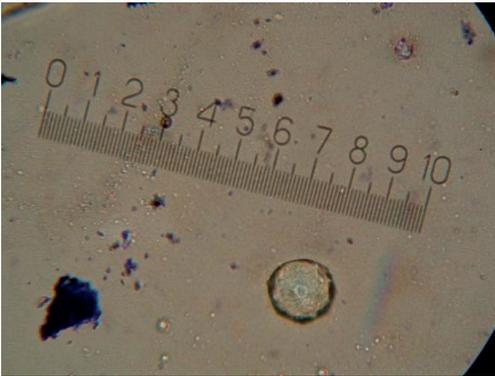


Figure 8. Periporate operculate pollen grains (~ 37.5 µm)

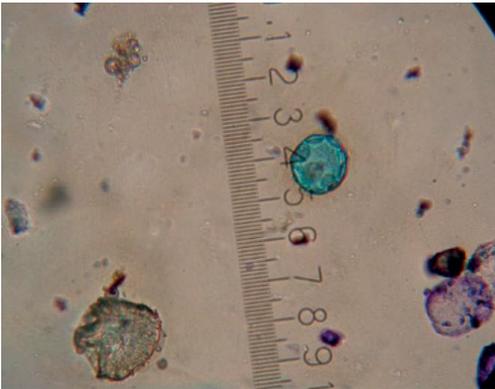


Figure 9. Periporate pollen grains (~ 37.5 µm), lenticular thickening around the pores can be seen

Small size tricolpate pollen grains were also noted (Figure 10), but not sufficient details were present on the pictures to be able to analyze them further.

Medium size tricolporate pollen grains possible *Fagus* (beech) and *Tilia* (lime tree) could also be found (Figures 11-12).



Figure 10. Tricolpate pollen grains (~ 20 µm)

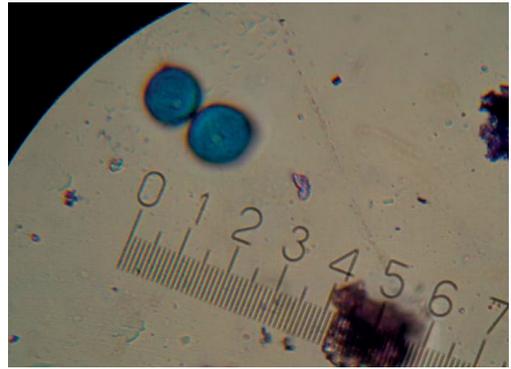


Figure 11. Tricolporate pollen grains with colpus and pore visible in lateral view (~ 30 µm)

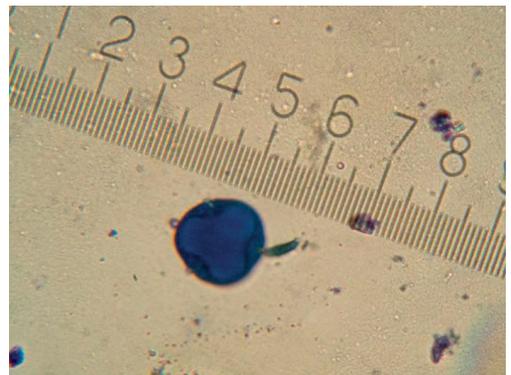


Figure 12. Tricolporate pollen grains with triangular convex polar shape with mid-wall apertures that have a thickening around them (~ 35 µm)

CONCLUSIONS

A simple pollen trapping experiment was used in the present study to analyze the morphology of some pollen grains from the air. Pinaceae pollen was over-represented on the slides, but several other pollen types were determined, reflecting the vegetation found in the immediate vicinity.

The inventory of plant species with allergenic potential in certain areas of the city and the knowledge of their flowering periods - the establishment of the pollen calendar, brings important data for pollen allergy sufferers, children, but also for the general population.

REFERENCES

Berger, U. (2020a). HNO Klinik der Medizinischen Universitaet Wien, Forschungsgruppe Aerobiologie und Polleninformation. *Aerobiology. Courses and Meetings*. Retrieved May 4, 2020, from

- <https://www.pollenwarndienst.at/AT/en/aerobiology/raining/courses-and-meetings.html>.
- Berger, U. (2020b). HNO Klinik der Medizinischen Universitaet Wien, Forschungsgruppe Aerobiologie und Polleninformation. Aerobiology. Methodology. Retrieved May 4, 2020, from <https://www.pollenwarndienst.at/AT/en/aerobiology/methodology/measurements.html>.
- Berghi, O. (2012). Răspândirea în România a plantelor anemofile cu potențial allergenic. *Viața medicală* 16 (1162). Retrieved February 26, 2018, from http://www.viata-medicala.ro/*articleID_5124-dArt.html.
- Bucher, E., & Kofler, V. (2020). Pollenatlas. In: Uwe Berger: HNO Klinik der Medizinischen Universitaet Wien, Forschungsgruppe Aerobiologie und Polleninformation. Aerobiology. Pollenatlas. Retrieved May 4, 2020, from <https://www.pollenwarndienst.at/AT/en/aerobiology/pollen-atlas.html>.
- D'Amato, G. (2007). Pollen allergy in Europe. The UCB Institute of Allergy – 09/2007. Retrieved May 11, 2020, from https://www.ucb.com/_up/_up/tuioa_com/images/PollenAllergy-DAmato-simplified-V2-070910_PP.pdf.
- Enache, M., Coman, M., Hangan, M. (2019). Initial steps towards the establishment of a pollen collection at USAMV Bucharest: the study of allergenic pollen. *Scientific Bulletin Series F: Biotechnologies, XXIII*, 260-266.
- European Aerobiology Society (2020). Project supports. Retrieved May 4, 2020, from: <http://www.eas-aerobiology.eu/eas-supports/>
- Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., Berger, U., Clot, B., Brandao, R., EAS QC Working Group (2014). Pollen monitoring: minimum requirements and reproducibility of analysis. *Aerobiologia*, 30, 385-395.
- Ianovici, N. (2007). The principal airborne and allergenic pollen species in Timișoara. *Annals of West University of Timișoara, ser. Biology, X*, 11-26.
- PalDat - a palynological database. Retrieved March 18, 2019, from www.paldat.org.
- Stebler, T. (2019a). Pollen-Wiki - Der digitale Pollenatlas. Retrieved March 31, 2019, from <https://pollen.tstebler.ch/MediaWiki/index.php>.
- Stebler, T. (2019b). Kategorien, Pollen-Wiki - Der digitale Pollenatlas. Retrieved May 12, 2019, from <https://pollen.tstebler.ch/MediaWiki/index.php?title=Kategorien>.
- Șerbănescu-Jitariu, G., Mitroiu-Rădulescu, N., Rădulescu, D. (1994). *Monografia polenului florei din România*. Vol. IV. Editura Academiei Române, București.
- Tarnavschi, I. T., Șerbănescu-Jitariu G., Mitroiu-Rădulescu N., Rădulescu D. (1981; 1987; 1990). *Monografia polenului florei din România*. Vol. I, II, III. Editura Academiei Române, București.