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INITIAL STEPS TOWARDS THE ESTABLISHMENT OF A POLLEN COLLECTION AT USAMV BUCHAREST: THE STUDY OF ALLERGENIC POLLEN

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Abstract

Pollen allergy (polinosis) is one of the most common allergic seasonal respiratory diseases in Romania. Allergenic pollen is generally anemophilous, it is produced in large quantities, it is light and can be transported by the atmospheric currents and it contains major allergens. The detection of aeroallergens, the inventory of plant species with allergenic potential and the knowledge of their flowering period provide valuable information for both allergy sufferers and allergy physicians. Thus, several European countries have developed aerobiological surveillance networks that make daily bulletins containing data needed to prevent exposure to local allergenic pollen. Considering the theoretical and practical importance of this subject and the current need to develop a network of aerobiological monitoring laboratories in our country, the aim of the present work was the morphological study of the pollen with allergenic potential found in Romania by microscope analysis and the establishment of a collection of pollen images at our laboratory. Such a collection is needed for comparisons and further identification of pollen grains from the air.

Key words*: pollen allergy (polinosis), allergenic pollen, optical microscope.*

INTRODUCTION

Pollen grains are the most important cause of outdoor allergies. The pollinic allergens are water-soluble proteins or glycoproteins found in the cytoplasm of the pollen grains but they can be released and after contact with the airway mucosa or the conjunctiva of allergic individuals specifically sensitised they can trigger an IgE antibody-mediated allergic reaction in seconds (Taketomi et al., 2006).

According to RNSA (2019) the type of pollinic allergen determines the allergenicity potential of a particular pollen, but the allergy risk is due to two more factors: the size of the pollen grain, since smaller pollen are lighter and will stay longer in the air and the quantity of the pollen that is released by a plant, since that influence the risk of exposure too.

During the last several decades airborne pollen and fungal spores started to be recorded permanently by special pollen monitoring stations with air sampling equipment distributed in numerous European contries (Thibaudon & Monnier, 2015). This analysis is coupled with the mapping of the allergenic pollen vegetation to help prevent environmental exposure which is important for the whole population, but mostly for allergy sufferers and atopic children. The aerobiological monitoring is based on the microscopic counting and identification of pollen grains at regular intervals and needs to be carried out by trained specialists (aerobiologists) who apply specific methods and standards (Galán et al., 2014; Garcia-Mozo, 2017). Because of the difficulties and the time consuming nature of this manual analysis, the aerobiological monitoring is carried out in certain centers only and is not able to provide information for the whole territory of a country.

According to Raţiu (1971) the research in aeropalynology has started in Romania in the 60s with the first iatropalynological study having the title "Determination of the degree of pollen infestation of the atmospheric air in Bucharest and the sensitizing value of some allergens prepared from pollen" (Bulla et al., 1963), this was followed by other studies (Seropian et al., 1963; Popescu et al., 1965; 1966; 1969; Capetti et al., 1969). Thus, it was found that 1-25% of the medically investigated allergies are pollinoses, being caused mainly by *Poaceae* pollen, by pollen of some *Asteraceae*, by poplar (*Populus*) and linden (*Tilia*) (Raţiu,

1971). Also, some studies indicated a higher incidence of polynoses in the sub-Carpathian regions, which have a different climate, but have similar vegetation represented by deciduous forests dominated by oak, beech, poplar, birch and other species, as well as plains with grasses, *Asteraceae*, *Plantaginaceae* etc. (Popescu & Capetti, 1971).

More recently, microscopic identification of pollen from allergenic species was carried out in Oradea area (Pallag et al., 2011) and the first centre for aerobiological study of pollen was opened at the Biology Department of the West University of Timișoara (1999-2012) (Ianovici & Faur, 2004; Ianovici, 2007a; 2007b; 2016) and the second at the Colentina Clinical Hospital in Bucharest (2014-2016) (Leru et al., 2018).

MATERIALS AND METHODS

The vegetation that produces allergenic pollen found in Romania includes species of trees, grasses and weeds and is spread throughout the country (Table 1) (Berghi, 2012). High allergenicity potential have the *Poaceae*, *Betula*, *Corylus*, *Quercus*, *Platanus*, *Ambrosia*, *Artemisia* and *Parietaria* (RNSA, 2019). *Poaceae* pollen is the most important allergenic pollen in Europe where it has a large distribution and there are high sensitisation rates among allergy suferers (McInnes et al., 2017). Working with allergenic pollen should follow specific safety regulations, any exposure, even if very small, entails some risk of sensitisation at any age.

The present study was carried out at the Laboratory of Biology of the Faculty of Biotechnologies, the University of Agronomic Sciences and Veterinary Medicine of Bucharest, using fresh plant inflorescences collected during spring 2018 and the herbarium of the laboratory.

The analysis of pollen from the herbarium: from the 12 species of *Poaceae* that are considered important due to their allergy risk, 4 species were found with flowers in the laboratory collection, namely: *Hordeum vulgare*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*; of the 8 weed genera that are considered important due to their allergy risk only two were found with flowers in the laboratory collection, namely *Plantago lanceolata* and *Urtica* sp. (*U. dioica* and *U. urens*). Instead of *Amaranthus* sp., *Celosia cristata* was used since its inflorescence was very well preserved, this genus has very similar pollen grains with *Amaranthus retroflexus* and with *A. graecizanus* (Tarnavschi et al., 1981, pp. 44).

Table 1 Grasses, trees and weeds that produce allergenic pollen found in Romania

	Plant family	Genus/species
Grasses	*Poaceae*	*Alopecurus pratensis* (meadow foxtail) *Anthoxantum odoratum* (sweet vernal grass) *Avena sativa* (oat) *Dactylis glomerata* (cock's-foot) *Festuca rubra* (red fescue) *Holcus lanatus* (meadow soft grass) *Hordeum vulgare* (barley) *Lolium perenne* (perennial ryegrass) *Phleum pratense* (timothy-grass) *Poa pratensis* (blue grass) *Secale cereal* (rye) *Triticum aestivum* (*T. vulgare*) (common wheat)
Trees	*Aceraceae*	*Acer* sp. (maple)
	Betulaceae	*Alnus* sp. (alder) *Betula* sp. (birch) *Carpinus* sp. (hornbeam) *Corylus* sp. (hazel)
	Cupresaceae	*Juniperus* sp. (junipers) *Thuya* sp.
	Fagaceae	*Castanea* sp. (chestnut) *Fagus* sp. (beech) *Quercus* sp. (oak)
	Juglandaceae	*Juglans* sp. (walnut)
	Moraceae	*Morus* sp. (mulberry)
	Oleaceae	*Fraxinus* sp. (ash) *Ligustrum* sp. (privet)
	Platanaceae	*Platanus* sp. (plane trees)
	Salicaceae	*Populus* sp. (poplar) *Salix* sp. (willow)
	Tiliaceae	*Tilia* sp. (lime tree/linden)
	Ulmaceae	*Ulmus* sp. (elm)
Weeds	*Asteraceae*	*Ambrosia elatior (A. artemisiifolia)* *A. trifida* (ragweeds) *Artemisia vulgaris* (mugwort) *Xanthium strumarium* *X. commune* (cockleburs)
	Amaranthacee	*Amaranthus* sp.
	Plantaginaceae	*Plantago lanceolata* (plantain)
	Polygonaceae	*Rumex* sp. (dock, sorrels)
	Urticaceae	*Urtica* sp. (nettle) *Parietaria officinalis* (common pellitory)

Pollen was analysed from fresh flowers in the case of *Acer pseudoplatanus, Betula verrucosa (B. pendula, B. alba), Corylus avellana, Fraxinus excelsior, Juglans regia, Ligustrum vulgare, Platanus* sp.*, Quercus robur, Salix caprea, Tilia* sp. and wild grasses.

Pollen wet mounts with or without staining (toluidine blue - TB) were analysed using an optical microscope Micros Austria. To measure, an ocular micrometer was used, the calibration ratio was 1 µm for ob. 100× and 2.5 µm for ob. 40×. Microscopic images of the pollen grains were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) and were later used to describe the grains. Comparisons were made with pollen descriptions found in the literature (Tarnavschi et al., 1981, 1987, 1990; Şerbănescu-Jitariu et al., 1994) or on various Internet sites (Pollen-Wiki - Der digitale Pollenatlas, Stebler, 2019a; the PalDat - Palynological Database or the Pollen Atlas of the Medical Faculty of Wiena, Berger, 2019). The classification of pollen according to size is from Stebler (2019b).

RESULTS AND DISCUSSIONS

A selection of images of the allergenic pollen grains analysed in the present study are presented in Figures 1-18. There were:

- triporate pollen grains: *Betula verrucosa*, *Corylus avellana*, *Urtica* sp.;
- tricolpate pollen grains: *Acer pseudoplatanus, Fraxinus excelsior, Platanus* sp.*, Quercus robur*;
- periporate pollen grains: *Juglans regia, Celosia cristata, Plantago lanceolata;*
- tricolporate pollen grains: *Ligustrum vulgare, Salix caprea, Tilia* sp.;
- monoporate pollen grains: *Poaceae.*

The triporate pollen grains of the two *Betulaceae* that were analysed have similar grain shapes, exine pattern and protruding pores (onci), however these are larger in *Corylus* than in *Betula* and in *Betula* a vestibulum is present (Figures 1-4). Birch pollen is small (~ 25 µm) while hazel has medium size pollen (~30 µm).

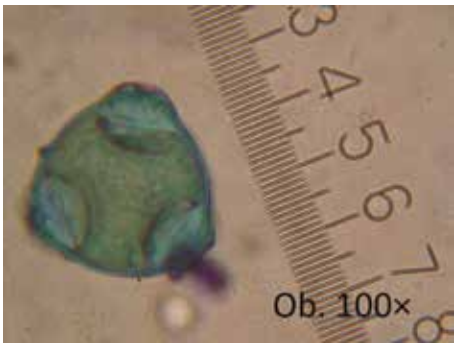

Figure 1. *Corylus* pollen, polar view (TB)

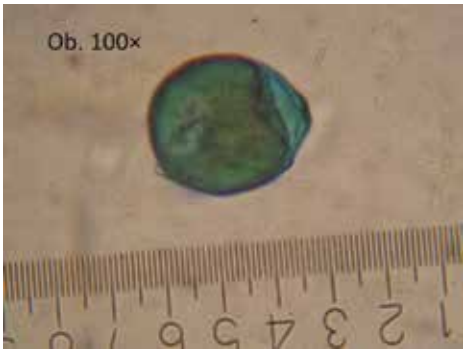

Figure 2. *Corylus* pollen, lateral view (TB)

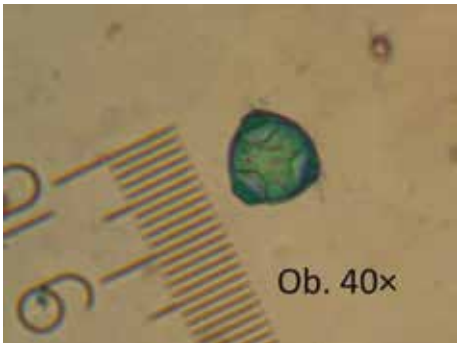

Figure 3. *Betula* pollen, polar view (TB)

Figure 4. *Betula* pollen, optical section (TB)

Triporate *Urtica* pollen (both *U. dioica* and *U. urens*) is similar to the pollen of *Parietaria,* and has small size and psilate exine (Figure 5).

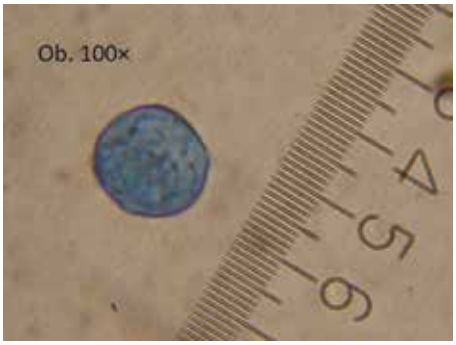

Figure 5. *Urtica* pollen, polar view (TB)

The four tricolpate pollen grains that were analysed (maple pollen, oak pollen, plane tree pollen, ash pollen) could be difficult to distinguish, they have similar polar and equatorial shapes and small to medium sizes (Figures 6-11). Therefore the colpi length, width, shape of the colpi apex and the surface pattern of the grain must be considered.

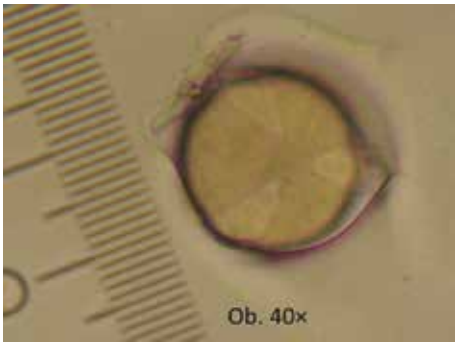

Figure 6. *Acer* pollen, polar view

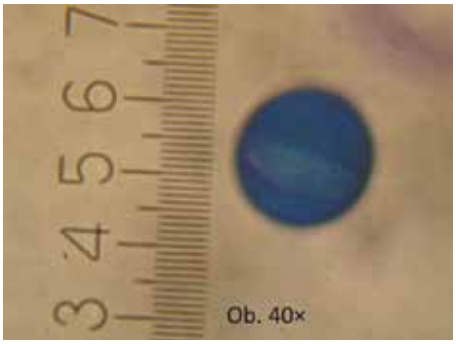

Figure 7. *Acer* pollen, lateral view (TB)

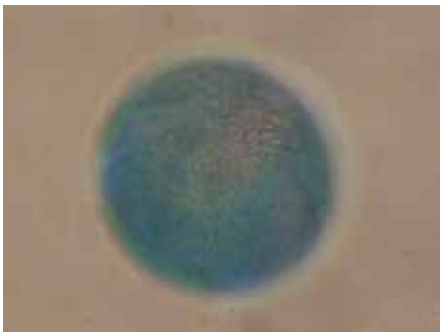

Figure 8. *Acer* pollen, striate exine (TB)

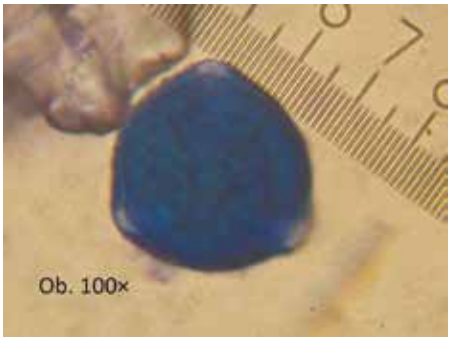

Figure 9. *Quercus* pollen, polar view (TB)

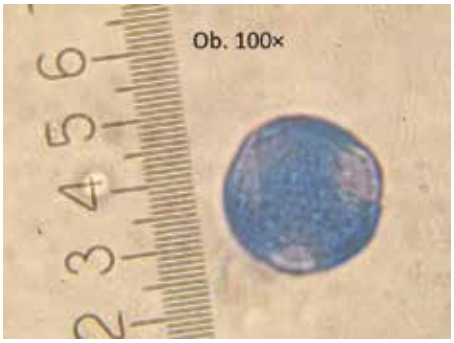

Figure 10. *Platanus* pollen, polar view (TB)

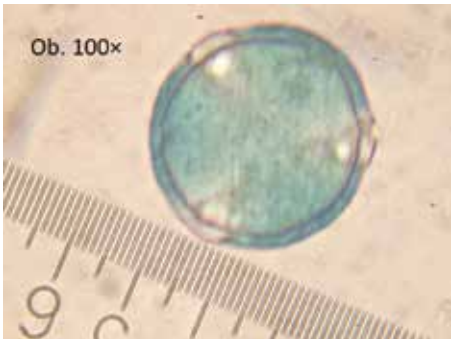

Figure 11. *Fraxinus* pollen, polar view (TB)

The periporate pollen grains that were analysed (*Juglans regia, Celosia cristata, Plantago lanceolata*) have a medium size and a spheroidal shape. Both *Plantago* and *Celosia* pollen are pantoporate (Figures 12, 13), but *Juglans* pores can be distributed unevenly and have a lenticular thickening around them (onci) (Figure 14).

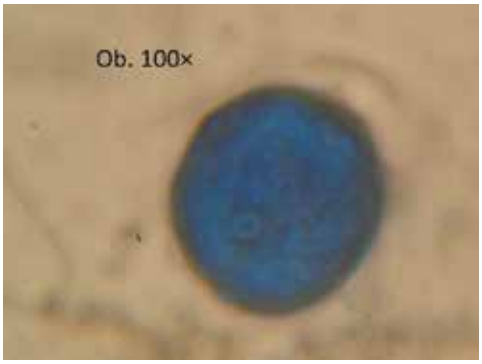

Figure 12. *Plantago* pollen, pantoporate, operculate (TB)

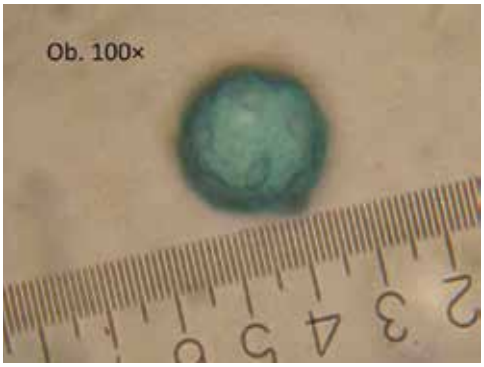

Figure 13. *Celosia* pollen, pantoporate, verrucate exine (TB)

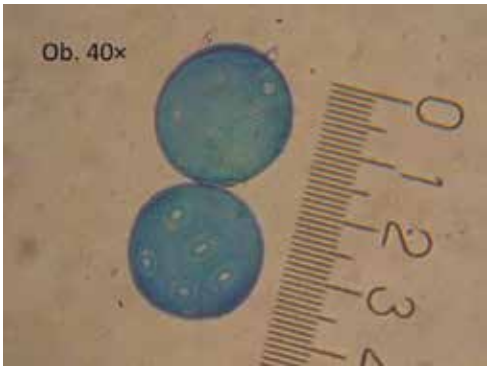

Figure 14. *Juglans* pollen, size > 40 µm (TB)

The analysis of the tricolporate pollen grains, namely *Ligustrum vulgare, Salix caprea* and *Tilia* sp. included grains that have different shapes and characteristics. The pollen of *Ligustrum vulgare* is medium size, has a triangular convex shape in apical view, it is oblate in equatorial view and on the surface has a reticulate pattern (Figure 15).

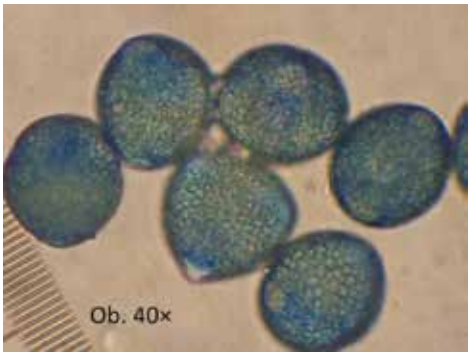

Figure 15. *Ligustrum* pollen, polar and lateral view, ~45 µm (TB)

Tilia pollen is medium size, the polar shape is triangular convex with mid-wall apertures that have a thickening around them, the equatorial shape is oblate (Figure 16).

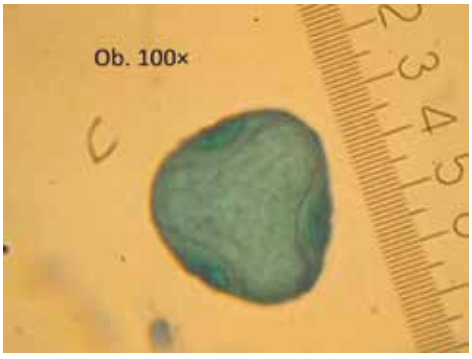

Figure 16. *Tilia* pollen, polar view, ~35 µm (TB)

Salix caprea has a small pollen, is circular with a reticular pattern (Figure 17).

The fresh *Poaceae* pollen, similar to the one from the herbarium showed the characteristics of the pollen of this family: monoporate, annulate, heteropolar and of small or medium size (only some cultivated *Poaceae* have large size pollen grains) (Figure 18).

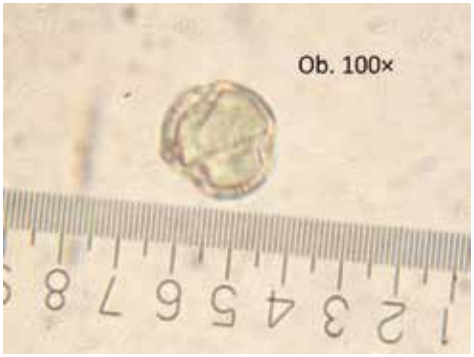

Figure 17. *Salix* pollen, ~18 µm

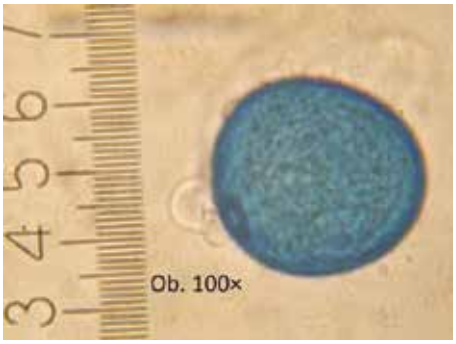

Figure 18. Medium size *Poaceae* pollen

CONCLUSIONS

Although the number of anemophilous plants is high, there are only about 20 allergenic pollen types that are being monitored. The knowledge and experience of their morphological forms alow that a visual pollen counting could be achieved at magnification 400×.

Knowledge of the potential allergy risk that some plants have is important in landscaping, especially in the case of trees and ornamental grasses, but also in the prevention of growth of invasive allergenic species such as *Ambrosia.*

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