

Morphological Research on Indigenous *Sambucus* Species Pollen

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Abstract

The pollen grains have a definite shape, size, colour, structure for each species, genus and family and these characters are useful for systematical botany. The pollen has nutritive properties due to its content: proteins, lipids, carbohydrates, vitamins, hormones and minerals. In the Romanian flora vegetate three species of *Sambucus*, but only *S. nigra* L. (elder or black elder) supplies a vegetal medical product, Sambuci flos or elder flowers, whereas the others species *S. ebulus* L. (dwarf elder) and *S. racemosa* L. (mountain elder or red elder) are considered adulterations. The pollen of *Sambucus* species were already studied using optical microscopy (Tarnavschi *et al.*), but the images are in one single layout, therefore the structure details cannot be easily notice. In this context the pollen grains of the three species already mentioned above were studied by SEM (Scanning Electron Microscopy). The results demonstrated that this pollen have a small-middle size, oblat-sphaeroidal-prolat shape, threecolpat and the exine adornments are of reticulate type, haemitectate with sticks in the meshes of polygonale net. The flavonoids content is lower than in others species (0.146-0.564 %). The SEM analyse of *Sambucus* pollen allow a reliable identification of the genus but less for the species.

Keywords: pollen, flavonoids, *Sambucus*, SEM (Scanning Electron Microscopy)

Introduction

The pollen grains, homologues to the ferns microspores, are produced in the pollenic bags of *Magnoliophytæ* (*Angiospermae*) anthers or in the male inflorescence (cone) of *Pinophytæ* (*Gymnospermae*). In the first stage these microspores are unicellulars, with haploid nucleus, then in maturation stage become twocellulars (a vegetative and generative cells) and are considered a male gametophyte. This will produce the two male gametes that are necessary for double fecundation in *Magnoliophytæ*, by the generative nucleus, after pollen germination upon female stigma of flowers (Ciobanu, 1971).

The pollen grains have a definite shape, size, colour, structure for each species, genus and family and these characters are useful for systematical botany (Ciobanu, 1971).

In the structure of mature pollen grains, two layers wall are distinguished, exine and intine that surround the two inner cells, one a bigger, the vegetative cell, and the other, smaller, the generative cell. Among the two layers of pollen wall, the exine is more developed, thickly and resisting in time, including sporopollenin polymer. Of this reason the pollen is useful in paleobotany and geological prospects (Dragastan *et al.* 1980, Tarnavschi, 1981).

The pollen grains contain proteins, lipids, carbohydrates, vitamins, hormones and minerals in the inner part,

whereas the others substances, like carotenoids and flavonoids are located in the external layer of wall. For this reason the pollen have a nutritive properties and this aspect was investigated after that the beekeepers managed to gather, by different technique, a greater quantities of pollen from the bees (Mărghitas, 2005). The bees used the pollen for their nourishment. In the medicine the pollen is studied in relation with allergy given of some sorts of pollen and, also, in legally medicine (Ialomiteanu, 1987; Subiza *et al.*, 1995; Kay, 1997; Mihele *et al.*, 2003).

The pollen of *Caprifoliaceae* family was studied by Tarnavschi *et al.* (1981) using optical microscopy and they consider that the pollen of this family is colporate, porat, subsphaeroidal, isopolar, tri-tetrasymmetric, of small-middle size and for *Sambucus* species the grains are 3-colporate, oblat-sphaeroidale and the exine without perpendicular thickness (Fig. 1). In the Romanian flora vegetate three species of *Sambucus*, but only *S. nigra* L. (elder or black elder) supplies a vegetal medical product, *Sambuci flos* or elder flowers, whereas the others species *S. ebulus* L. (dwarf elder) and *S. racemosa* L. (mountain elder or red elder) are considered adulterations (Tamas, 1999).

The aims of this study was to obtain the images of the *Sambucus* pollen's by SEM (Scanning Electron Micros-

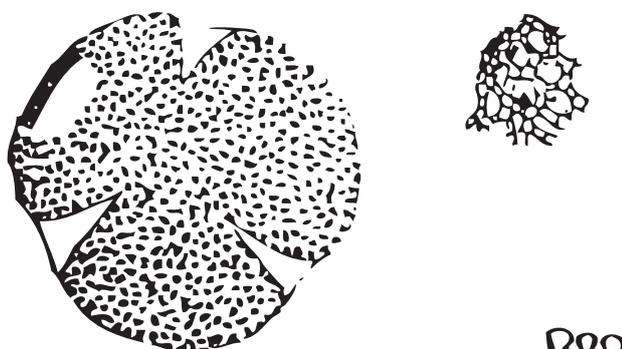
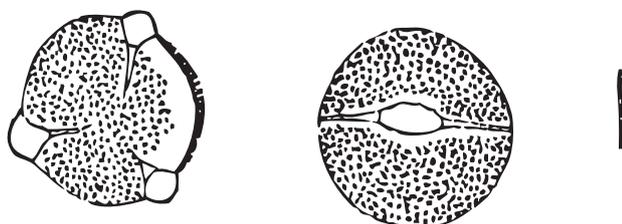
*Sambucus ebulus**Sambucus racemosa**Sambucus nigra*

Fig. 1. Designs for pollen grains of *Sambucus* analyzed species (by Tarnavski *et al.*, 1981 copy), for a define characterization and to establish the possible differences and similitude between these three species (*Sambucus nigra* L., *Sambucus ebulus* L., *Sambucus racemosa* L.) (Soran, 1971). Another objective was to analyze the flavonoids, a pharmacological active principles from *Sambucus* pollen in comparison with others species (Tamas, Hodisan, 1986).

Materials and methods

The pollen of the three species of *Sambucus* was gathered from inflorescences, dried on separate papers for a week. The inflorescences of *S. nigra* L. (elder) and *S. ebulus* L. (dwarf elder) were gathered from Cluj-Napoca City environs, while that of *S. racemosa* L. (mountain elder) from Băișorii Mountain (Cluj County).

The microscopic analyses were performed at a Scanning Electron Microscope JEOL JSM-5510 LV, after a vacuum metalizing technique (Ploaie, 1979). The SEM uses an electron beam to give a "photographic" image of the sample, but cannot provide quantitative data regarding the surface. SEM requires the sample to be covered with a very thin layer of a conductive material (gold of 10 nm thickness) and needs to be operated under vacuum during both metallization and measurements (Bhushan, 2004).

The qualitative analysis of flavonoids was made by TLC technique (York, 1990, Tamas, 2002) and the quantitative determination of flavonoids by a spectrophotometric method (Romanian Pharmacopeia Xth Edition, 1993).

Results and discussions

Following the images of SEM were calculated the polar (p.a.) and equatorial axis (e.a.), (Fig. 2-4 and Tab. 1) of pollen grains of *Sambucus*. In accordance with the dimensions and p.a./e.a. ratio, the pollen grains of *Sambucus* are framed in the prolat type (Tarnavski, 1981 p.a./e.a.=8/6-8/4) and after the size of pollen grains may be framed in the small size type (10-25 μm) and only that of *S. ebulus* have over 25 μm and may be framed in the middle size type (25-50 μm).

In accordance with the ratio p.a./e.a. (Tab. 1) the pollen grains have an ellipsoidal shape with 3 ditches (tricolpate type) namely oblat-spaeroidale. The sizes of *S. nigra* and *S. ebulus* are very close, the same for p.a./e.a. ratio (1.88-1.89), whereas for *S. racemosa* both, the sizes and ratio (1.53) are smaller than the formers, in *S. racemosa* the equatorial axis being greater.

In respect to exine adornment the pollen grains of *Sambucus* are of reticulate type with the polygonal mesh of the net and with a point in these meshes. The explanation of this appearance is in accordance with Tarnavski (1981), for the external layer of exine, namely sexine, the sculptural zone of exine (Fig. 5).

This is a haemitectat or subtectat type and give a reticulate structure of the exine while the points from the meshes of the net, represent the sticks or „bacculae" with pillar shape in cross section, whereas in surface view have a points shape.

Therefore, if in optical microscopy the images are in one single layout, in scanning electron microscopy, the images are three dimensional or spatial (Reille, 1992). The oval form, the structure details, the reticulate, tricolpate appearance and the sticks in the net's eyes can be very easily noticed. Furthermore, the images are standardized in micrometers, which allow the calculation of the dimension of the grains and details.

Regarding the flavonoids analysis, by mean of TLC, these prove the presence of flavonoidic compounds, with yellow-orange fluorescence in UV light and polyphenocarboxylic acids with blue fluorescence. With the aid of standard substances, the caffeic and chlorogenic acids were

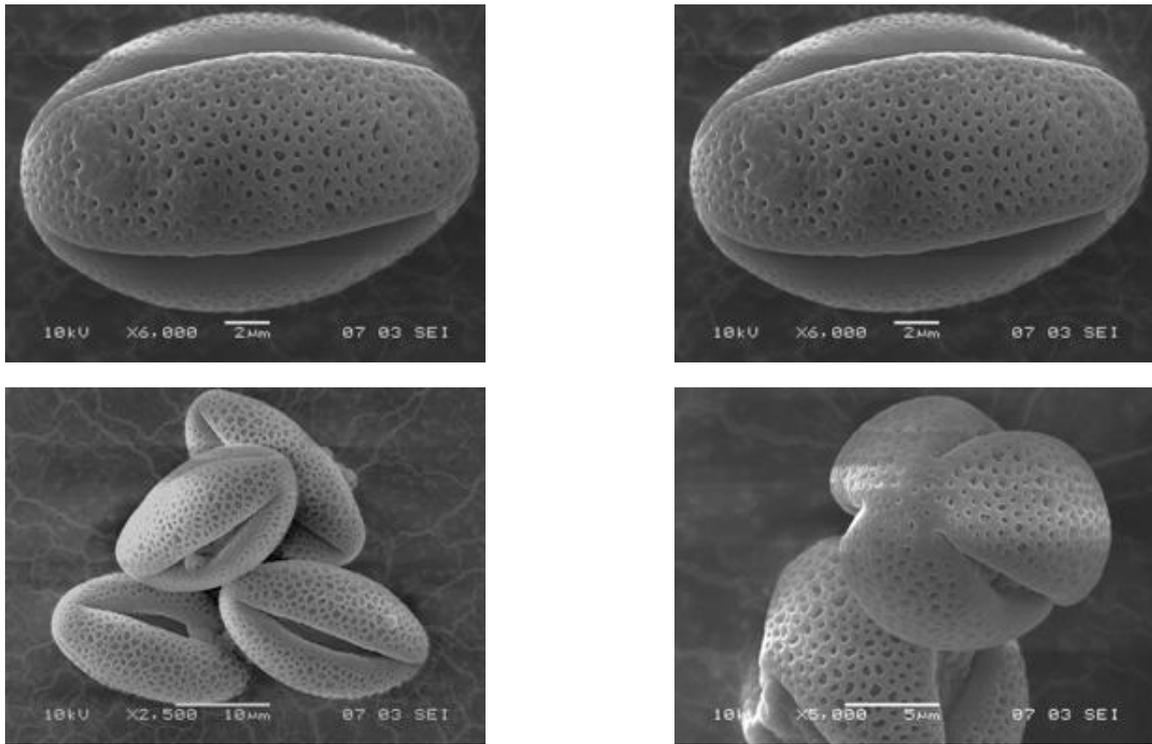


Fig. 2. SEM images for pollen grains – *S. racemosa*

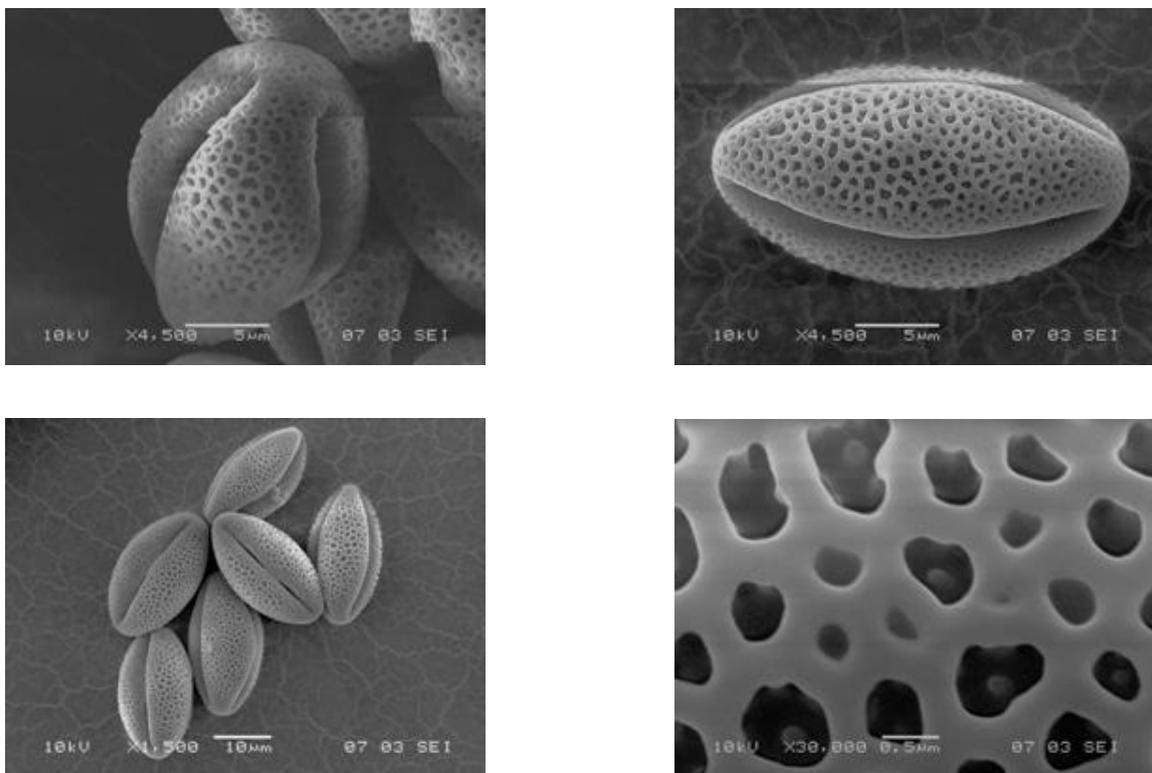
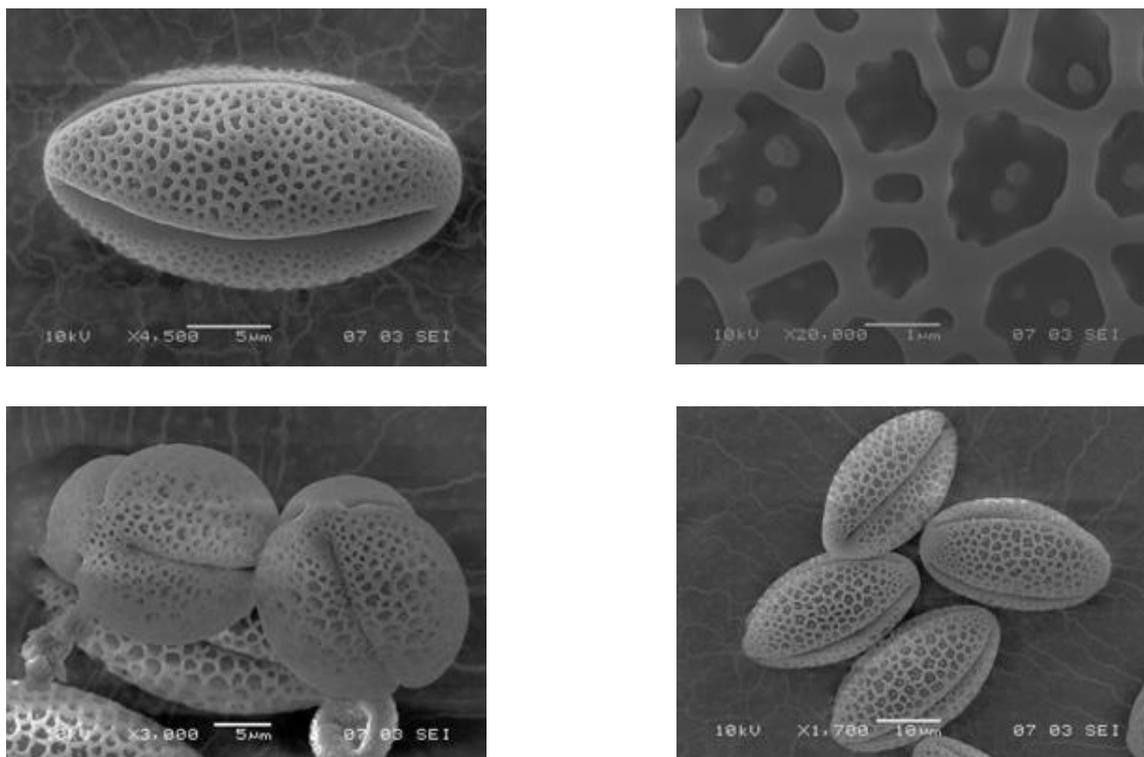


Fig. 3. SEM images for pollen grains – *S. nigra*

Fig. 4. SEM images for pollen grains – *S. ebulus*Tab. 1 Values of polar axis, equatorial axis and P/E ratio calculated for the three species of *Sambucus*

| Species | Polar axis (a.p.) (µm) | Equatorial axis (a.e.) (µm) | P/E (a.p./a.e. ratio) |
|--------------------|------------------------|-----------------------------|--------------------------|
| <i>S. nigra</i> | 24.25 | 12.85 | 1.88 |
| <i>S. ebulus</i> | 29.15 | 15.41 | 1.89 |
| <i>S. racemosa</i> | 20.00 | 13.06 | 1.53 |

identified, but the flavonoidic glycosides, especially rutoside, are in the traces, in comparison to the pollen of others species like walnut tree or hornbeam (Tamas, 1986).

The content of favonoids in *Sambucus* pollen shows a low level: 0.146 % for *S. nigra* pollen, 0.245 % for *S. ebulus* and 0.564 % for *S. racemosa*, whereas in hornbeam and walnut tree is 1.20% and, respectively 1.36%.

The facility of extraction for flavonoids with methanol confirm the hypothesis that the flavonoids are placed in the external layer of pollen wall, according with Wierman *et al.* (1983), Ritscher *et al.* (1983), Prahl *et al.* (1985) finding.

Fig. 5. Design of exine adornment (reticulate type) for *Sambucus* species (by Tarnavski *et al.*, 1981)

Conclusions

SEM offers better images for pollen grains concerning particular structure of exine comparatively with optical microscopy.

The pollen grains of *Sambucus* species have a reticulate adornment, an ellipsoidal shape (prolat), tricolpate and a small-middle size.

The pollen grains of *S. nigra* and *S. ebulus* are a very close in respect of size and shape whereas pollen of *S. racemosa* differs in respect of size, being smaller and in respect with proximale/equatoriale ratio.

The content of flavonoids in *Sambucus* pollen is lower than in others species (e.g. hornbeam or walnut tree), but are present polyphenolcarboxylic acids.

SEM analyses allow the accuracy identification of the pollen for *genus Sambucus* but not for the species of this.

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