

COMPARATIVE STUDY OF LIGHT MICROSCOPY IMAGES OF POLLEN FROM 3 ONAGRACEAE SPECIES

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Abstract

Due to their morphological characteristics such as: tetramerous, actinomorphic or zygomorphic flowers that are arranged in racemes, spikes, or panicles, simple, opposite or alternate leaves, lanceolate in shape, a capsule or, rarely, a berry fruit, very small seeds that have in some genera, tufts of hairs, the Onagraceae species are considered easy to recognize in the field. Some of the species found in Romania are well known melliferous plants, popular ornamental plants, ruderal plants or plants that have been used in traditional medicine. Different genera have pollen grains that have different morphological characteristics such as the shape, size, the apertures and the surface patterns, but in many genera they are held together by viscin threads that make the pollen sticky. Although modern techniques of pollen analysis include electron microscopy, most pollen studies also use conventional light microscopy. In the present study, images of hydrated pollen from *Fuchsia magellanica* 'Alice Hoffman', *Oenothera biennis* (common evening-primrose) and *Epilobium parviflorum* (small-flowered willowherb) were provided by light microscopy and were compared to the descriptions found in the literature.

Key words: *Epilobium parviflorum*; *Fuchsia magellanica*; light microscope; microphotographs; *Oenothera biennis*; pollen morphology.

INTRODUCTION

In Romania, the Onagraceae (Oenotheraceae) family - known also as the evening-primrose family, includes 27 spontaneous and cultivated species, that belong to 6 genera: *Chamaenerion* (*Chamaenerion*), *Circaea*, *Epilobium*, *Fuchsia*, *Ludwigia* and *Oenothera* (Ciocârlan, 2009).

Fuchsia is a very popular ornamental, perennial, container plant, grown mostly outdoors during summer for its dozens of multi-colored pendulous flowers with teardrop shape that bloom from spring until fall, usually if sheltered from intense sun and heat.

A ruderal biennial plant that is found growing in sunny places in the sandy soils of the river meadow is *Oenothera*. *Oenothera biennis* (*Onagra biennis*) (common evening-primrose) has large yellow flowers that open fast every evening and is sometimes cultivated in gardens or parks for its horticultural value. The mature seeds contain gamma-linolenic acid that has medicinal uses. *Oenothera* is melliferous,

attracting pollinators such as moths, butterflies, and specialized bees.

Some *Epilobium* and *Chamaenerion* species are also melliferous: *Epilobium parviflorum* and *Chamaenerion angustifolium* (*Epilobium angustifolium*), are herbaceous, perennial plants, found all over the country. Due to their use in traditional medicine, the aerial parts of some *Epilobium* species have been used as herbal tea.

The morphology of the Onagraceae pollen grains was investigated in numerous studies, for example Brown (1966) (also see Perveen & Qaiser, 2013 for literature citations). Moreover, pollen development was described for selected Onagraceae species (Takahashi & Skvarla, 1990; Rowley & Skvarla, 2006; Rowley & Skvarla, 2007).

The study of pollen uses the light microscopy for counting and identifying pollen grains of a particular sample and scanning electron microscopy for morphological comparisons and taxonomy (Jones & Bryant, 2007).

In the present study, light microscopy images were used for three species, each from a different Onagraceae genera (*Fuchsia*, *Oenothera* and *Epilobium*) to analyse the hydrated pollen grain and to compare the results to the descriptions found in the literature (Brown, 1966; Halbritter, 2016; Auer, 2020; Halbritter & Auer, 2020; Stebler, 2021b, c, d).

MATERIALS AND METHODS

Pollen of *Epilobium parviflorum* was analysed from dry plant material (*Epilobii herba*) obtained commercially, while pollen of *Oenothera biennis* and *Fuchsia magellanica* 'Alice Hoffman' was analysed from fresh plants cultivated in Bucharest (Figures 1-3).

The microscopic analysis was carried out in the Laboratory of Biology, Faculty of Biotechnologies of the University of Agronomic Sciences and Veterinary Medicine of Bucharest using wet mounts (sometimes toluidine blue (TB) was added for better contrast) and a Micros Austria optical microscope with ocular micrometer (calibration ratio was 1 μm for objective 100 \times and 2.5 μm for objective 40 \times).

Microscopic images were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5 \times zoom lens).

The classification of pollen according to size is from Stebler (2021a).



Figure 1. *Epilobii herba* used in the present study



Figure 2. *Fuchsia magellanica* 'Alice Hoffman' used in the present study



Figure 3. *Oenothera biennis* used in the present study

RESULTS AND DISCUSSIONS

Epilobium parviflorum pollen showed large, 3-zonoporate, oblate pollen grains with protruding apertures, psilate surface and viscin threads (Figures 4-5).

The pollen of *Fuchsia magellanica* 'Alice Hoffman' showed large, 3-zonoporate pollen grains with slightly protruding apertures,

scabrate surface and viscin threads (Figures 6-8).

Oenothera biennis pollen images show large, 3-zonoporate, oblate pollen grains with distinctly protruding apertures, scabrate exine, a wrinkled surface of the polar area in grains that are not fully hydrated and viscin threads (Figures 9-13).

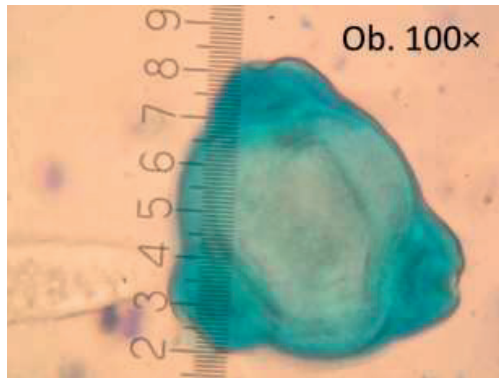


Figure 4. *Epilobium parviflorum* pollen grain with circular outline in polar view (TB)

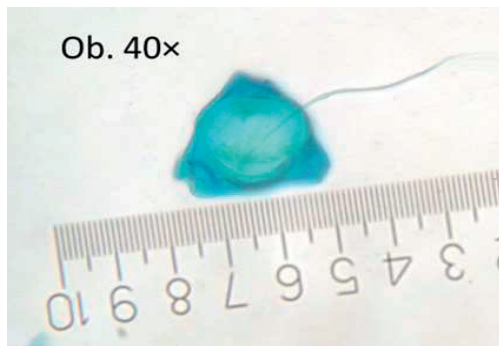


Figure 5. *Epilobium parviflorum* pollen grain in slightly oblique polar view, viscin thread visible (TB)

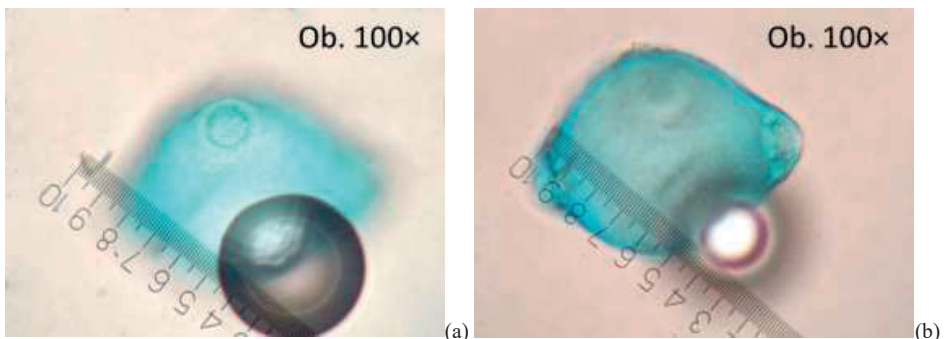


Figure 6. *Fuchsia magellanica* 'Alice Hoffman' pollen grain in slightly oblique equatorial view, (a) upper focus, (b) optical section (TB)

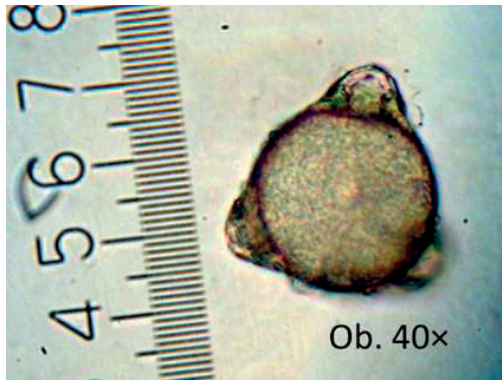


Figure 7. Pollen grain of *Fuchsia magellanica* 'Alice Hoffman' - exine surface

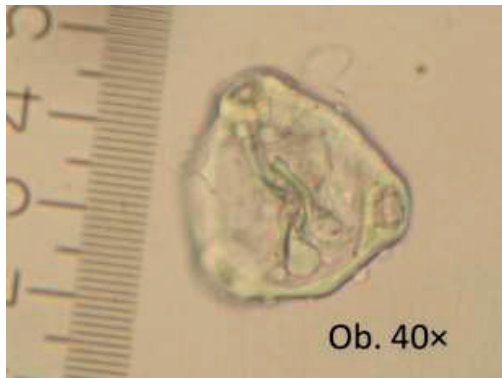


Figure 8. Pollen grain of *Fuchsia magellanica* 'Alice Hoffman' - viscin threads are visible

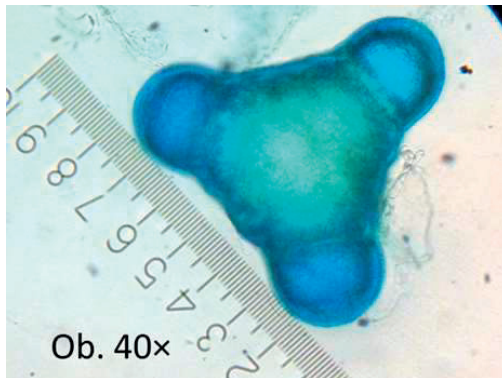


Figure 9. *Oenothera biennis* pollen grain with triangular outline and protruding apertures at each corner in polar view (TB)

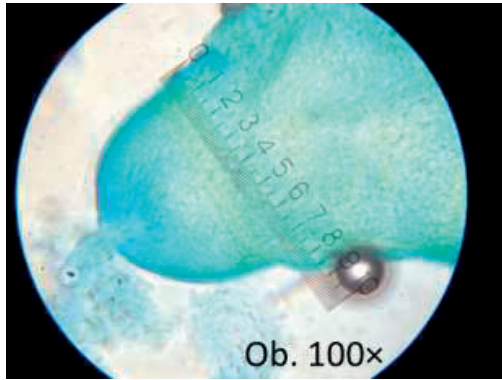
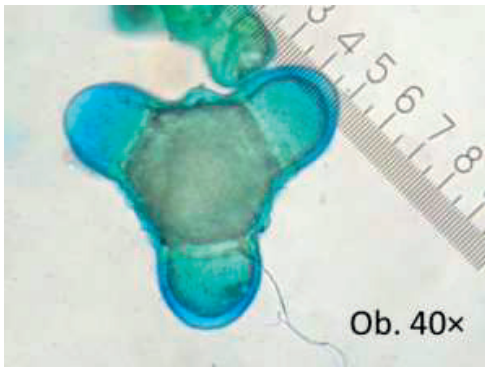
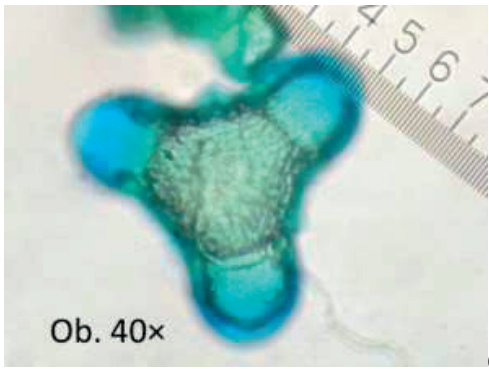


Figure 10. *Oenothera biennis* pollen grain - protruding aperture $\sim 45 \mu\text{m}$ deep and $67 \mu\text{m}$ wide; exine $\sim 3 \mu\text{m}$ thick (TB)



(a)



(b)

Figure 11. *Oenothera biennis* pollen grains in polar view – (a) optical section, (b) upper focus, distinct areas at the junctions with the protruding apertures are visible (TB)

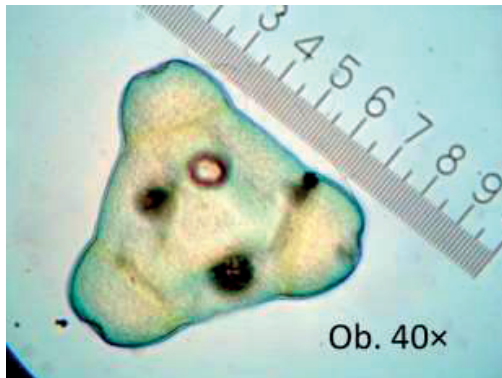


Figure 12. Pollen grain of *Oenothera biennis* - exine surface

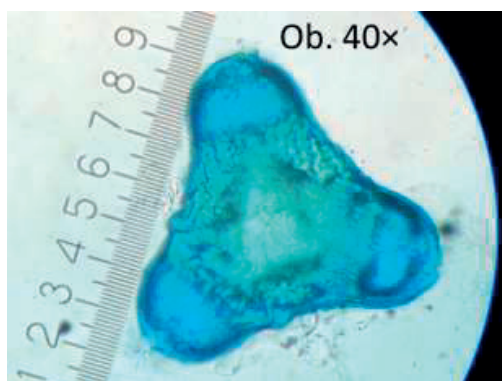


Figure 13. *Oenothera biennis* pollen grain showing a wrinkled surface of the polar area (TB)

CONCLUSIONS

The three Onagraceae species that were analysed in the present study belong to three different genera and they have a few different characteristics, such as the outline in polar view and the distinct apertural protrusions, that allow them to be distinguished in light microscopy images. The data is similar to that found in the literature, however, *Epilobium parviflorum* is shed in tetrad, but here, when dry herbal tea was used as plant material, it was seen as single grain.

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