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PRELIMINARY ANALYSIS OF 8 BEE POLLEN SAMPLES COLLECTED AT THE MOARA DOMNEASCĂ EXPERIMENTAL STATION DURING AUTUMN 2021

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Abstract

The present study describes the results of a pollen analysis carried out using light microscopy of 8 bee pollen samples collected from apiaries established within Moara Domnească experimental station (Ilfov County) during the fall of 2021, after a summer characterized by very hot and dry weather conditions, especially during the months of June and July. The preliminary analysis indicated buckwheat pollen (Fagopyrum esculentum, Fam. Polygonaceae) as predominant in the pollen grains that were analyzed, then it indicated pollen similar to that of sunflower (Helianthus annuus), but also found in other Asteraceae species (Senecio type pollen) and in smaller amounts Crepis type pollen (can be weeds in crops or ruderal plants: Taraxacum, Leontodon, Cichorium, Tragopogon), Fabaceae-like pollen (Trifolium sp.) and Cirsium type pollen (can be Cirsium arvensis - weed in crops, but also other plants). In Romania, there are few areas cultivated with buckwheat, mostly in the northern part of Moldova. However, this is a fast-growing annual that can be grown as a late-season melliferous cover crop, whose flowers provide both pollen and nectar for honey bees and other pollinators.

Key words*: autumn pollen, bee pollen, buckwheat, optical microscope, pollen morphology.*

INTRODUCTION

Moara Domnească village is situated along the Pasărea river in Găneasa commune (Ilfov county, Muntenia region, Romania). The region's natural vegetation of steppe and silvosteppe has been largely replaced by agricultural crops.

Buckwheat (*Fagopyrum esculentum*) belongs to the Polygonaceae family. The species is included in the cereal group (pseudocereal) due to the chemical composition of the grains and their uses in food and feed. It is also a species with promising melliferous potential. Buckwheat has a short vegetation cycle of 90-120 days and a thermal requirement during the vegetation period of 15-18°C. The seeds germinate quickly, with emergence occurring 4-5 days after sowing, if the humidity in the soil is optimal and the temperature exceeds 10°C. Flowering in buckwheat begins 30-35 days after sowing and lasts about 4 weeks.

During the summer of 2021, within the Moara Domnească experimental station, an apiary comprising of 5 beehives was placed, and in the vicinity a buckwheat culture was established starting with August 20, 2021 (Figure 1).

Figure 1. The apiary in the Moara Domnească experimental station in October 2021

Since nectar secretion is influenced by a multitude of internal and external factors, some direct and indirect methods were used to determine the nectariferous capacity of plants (Drăgan et al., 2022). To evaluate the preference of pollen sources by the honey bee colony, 8 samples of pollen loads were

collected by the beekeeper using pollen traps and the pollen was analysed together, therefore an estimate of the integral melliferous character of the tested crop was possible.

MATERIALS AND METHODS

In the third decade of September 2021, the buckwheat crop was at the beginning of the flowering process (Figure 2), and on September 30, 2021, determinations were made on the crop density, the number of inflorescences/plant and the number of flowers per inflorescence.

Figure 2. Buckwheat experimental plot at the Moara Domnească experimental station (Ilfov County) on September 30, 2021

The analysis of bee pollen was carried out at the Laboratory of Biology of the Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The samples were kept in the freezer until the time of microscopic analysis. An M7A Wild Heerbrugg stereomicroscope and a Micros Austria optical microscope equipped with an ocular micrometer were used, the value of the gradations for each of the objectives used being listed in Table 1.

Table 1. The values of the micrometric graduations for the Micros Austria microscope used in the present work

Ob. 10×	Ob. 40×	Ob. 100×
10 µm	2.5 µm	1 µm

The methods used for making microscopic preparations used in pollen analysis are described in the specialized literature (for example Campos et al., 2021). In the present work, an estimate of pollen frequencies (Louveaux et al., 1978) was carried out using fresh preparations with or without toluidine blue staining (Enache et al., 2019). In order to identify the botanical origin of the pollen, microscopic images were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) and the images were compared with descriptions found in the literature (Halbritter & Auer, 2021; Halbritter et al., 2020; Raine et al., 2022; Stebler, 2024a; 2004b; 2004c; Tarnavschi et al., 1981).

RESULTS AND DISCUSSIONS

Since the colour of the pollen pellets has not been precisely determined, the results are mainly based on the size and other morphological characteristics of the pollen grains. The pollen grains from which some pellets are made up have the same morphological characteristics, suggesting that there may actually be only 5 different types of pollen, i.e. only 5 botanical sources, as follows: very frequent are light green pollen pellets (Figure 3), pollen grains are tricolporate, have large size (~59-62 µm P, ~32-45 µm E), prolate shape, are oval in lateral view and trilobate/circular in polar view, have reticulate ornamentation and thick exine (~ 2.5-3 µm), this description corresponds to buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae) (Figure 4).

Figure 3. Numerous light green pollen pellets, possible buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae) analysed in the current study

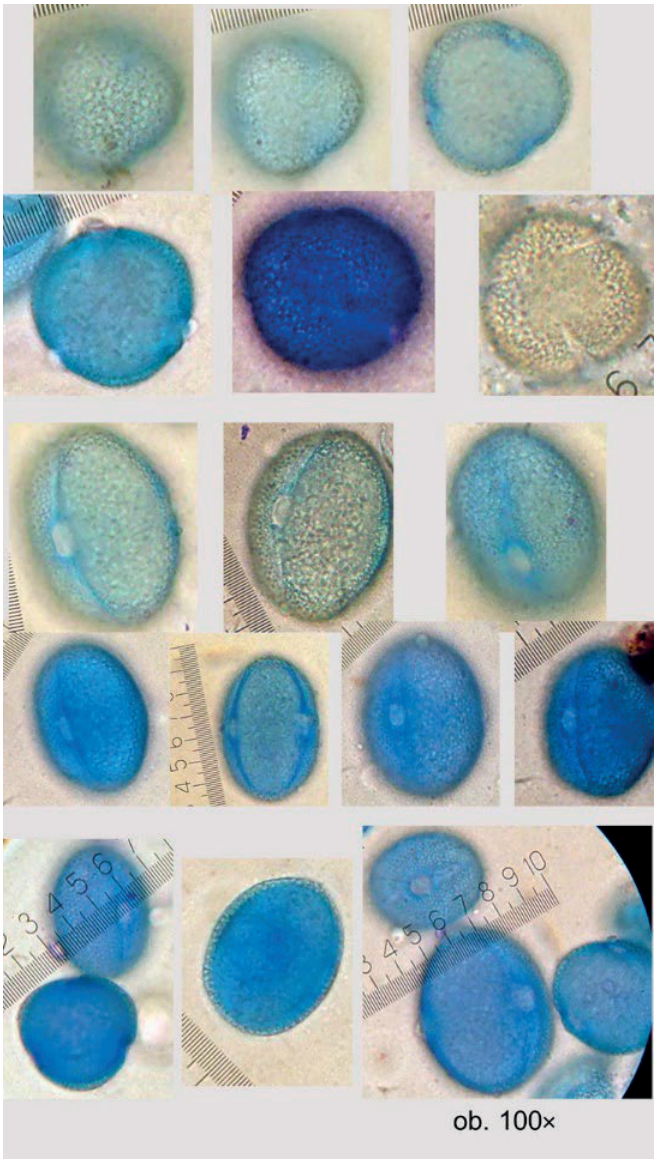

Figure 4. Tricolporate, prolate, large size pollen grains with reticulate ornamentation, possible buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae)

Frequent orange-yellow pollen pellets were seen. In this case, pollen grains are tricolporate, have medium size (~32 µm P, ~29 µm E), are spheroidal or oblate-spheroidal in shape, are echinate, with ~5-6 µm long echini, similar to sunflower pollen (*Helianthus annuus*), but found in other Asteraceae as well (Figure 5).

But also, some orange-yellow pellets are made of pollen grains that are 3-aperturate, fenestrate, echinate, with medium size (30-35 µm), could be ruderal Asteraceae (Figure 6).

In the images of Figure 7, from rare beige pollen pellets, pollen grains are tricolporate, have medium size (35-36 µm P, ~33-36 µm E), prolate-spheroidal shape, they are triangular convex in apical view, and have reticulate ornamentation, possible Fabaceae pollen (possible *Trifolium pratense*, red clover).

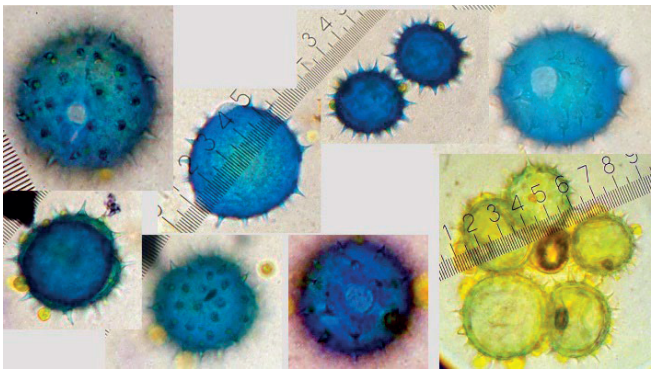

Figure 5. Tricolporate, spheroidal, medium size pollen grains with echinate ornamentation, similar to sunflower pollen (*Helianthus annuus*, fam. Asteraceae)

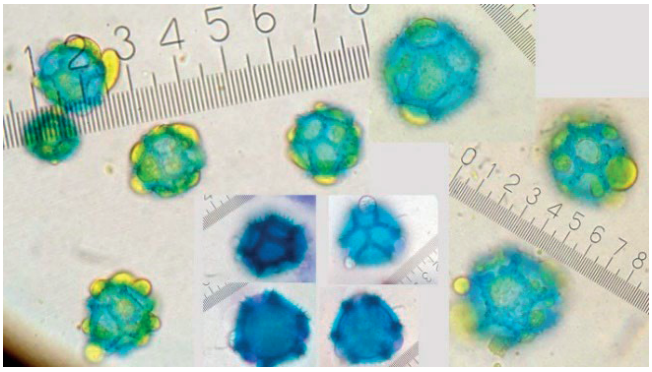

Figure 6. Triaperturate, fenestrate, echinate, medium size pollen grains, possible from ruderal Asteraceae

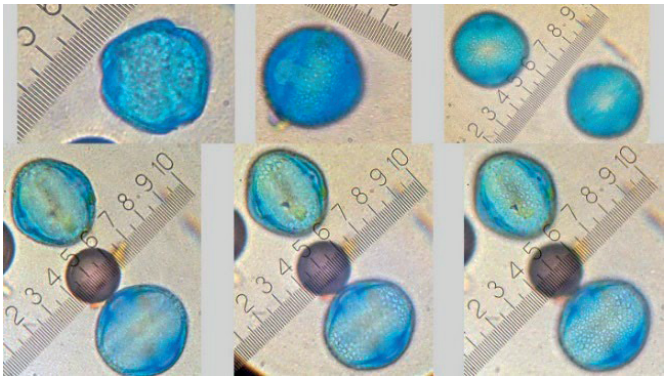

Figure 7. Tricolporate, prolate-spheroidal, medium size pollen grains with reticulate ornamentation, possible Fabaceae pollen

Rare black/dark brown pollen pellets were also found, consisting of pollen grains similar to those of *Cirsium* sp., fam. Asteraceae, possible ruderal Asteraceae (Figure 8). Pollen grains are tricolporate, echinate, have medium size (~ 40-45 µm P, ~ 45-50 µm E), oblate-spheroidal shape, the surface of the echini is not smooth, but has granules.

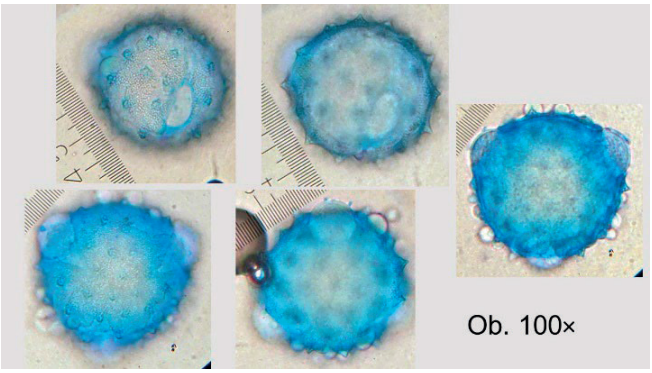

Figure 8. Tricolporate, oblate-spheroidal, medium size pollen grains with echinate ornamentation, possible from ruderal Asteraceae

CONCLUSIONS

For the period that was analysed (end of September-October 2021) the results showed: the monofloral source of pollen pellets; the importance of buckwheat crop (*Fagopyrum esculentum*, Fam. Polygonaceae) and other pollens of ruderal or cultivated plants present in the area (Asteraceae, Fabaceae).

Although in Romania in October there were generally no more flowering sources to collect, and the bees rarely came out of the hives, it is observed that currently the tenth month of the year, has sometimes warm days that alternate with cold and rainy periods, and in some years, especially in the south of the country, it is a month with summer temperatures and clear skies.

Thus, it can be observed that for the fall of 2021, a late harvest has been achieved, the exploitation of natural food resources continued in the month of October. Unfortunately, in the southern part of Romania, after the artichoke and sunflower flowers pass, the plains become unattractive for honey bees, due to the lack of honey plants (Ion, 2012).

The use of pesticides has contributed towards a decrease in the biodiversity of agricultural ecosystems, thus decreasing the number of honey plants. In this context, the results obtained within the project "AGROAPIS - Project for raising the value of beekeeping production by using agricultural crops beneficial to bees and pollinators in compliance with agro-environmental conditions" are particularly important.

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