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The Role of Pollen Analysis in the Sustainable Development

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Pollen analysis is becoming increasingly important in various scientific fields. In this article, the diversity of plants that produce pollen and bloom simultaneously with acacia was examined based on the pollen analysis of acacia honey samples. These samples, dating back to the 2000s, were collected from the same agricultural area. In the 19 honey samples examined, a total of 51 different species were identified, of which 40 species provide nectar, or both nectar and pollen and 11 species provide only pollen. The composition of the samples changed significantly over the years, both in quality and quantity. During the study period, the number of identified species increased. In relation to agricultural production, up to the year 2007, the pollen of *Trifolium pratense* was present at a high ratio (18.5 %), then it almost completely disappeared from the samples and was replaced by pollen from the *Brassicaceae* family at a higher ratio (22.9 %).

1. Introduction

Biodiversity and sustainability connect to each other in different ways. Biodiversity has been described as one of the major pathways to sustainability, and the protection of biodiversity is one of the basic roads to sustainability (Dikmenli, 2010). The diverse ecosystem is generally stronger in withstanding environmental stress and is likely to be more stable (Ngan et al., 2022).
With the increasing human population and the challenge of sustainable development, the world is increasingly reliant on pollinating insects, including bees (Plot and Boutillon, 2022). To ensure the pollination of fruits, vegetables, and agricultural crops, both for economic returns and to provide essential nutrients for human consumption), the importance of bees is paramount (Isaacs et al., 2017).
Beyond chemical composition analysis, pollen analysis (melissopalynology) plays a crucial role in assessing the quality of different mono- and polyfloral honey samples (Bobiş et al., 2020). This method can determine the botanical origin and geographical source of honey samples, as well as the degree of fermentation. Moreover, it holds economic significance as it can provide a solution to one of today's major problems, honey adulteration.
Honey always contains a variety of pollen grains, some adhering to bees' bodies during nectar collection, and others collected by bees in separate clumps - mostly used for feeding larvae. The pollen is regarded as a very valuable feed; it contains high protein and other valuable nutrients (Zuluaga et al., 2015). Moreover, the extremely high level of bioactive compound content entitles to industrial extraction (Salazar-González et al., 2019). The shape, size, and pattern of pollen are uniquely characteristic of the plant it comes from. All types of naturally sourced honey must contain an appropriate number of pollen grains, and the predominant pollen from the nectar source, called "leading pollen", must be present. Pollen analysis plays a significant role in certifying honey quality, setting numerical limits for economically significant types of honey in relevant standards (MSZ 6950-3).
Acacia honey holds great value due to its sought-after qualities: light colour, lasting liquid state, and delicate floral aroma. It is one of the most recognized types of honey in the European Union (Schievano et al., 2019). Acacia honey possesses distinct characteristics: the flowers have high nectar content but contain little pollen (Farkas and Zajácz, 2007). Therefore, the European protocol for monofloral honey defines the amount of acacia pollen alongside the total pollen count. Additionally, acacia honey has a higher sucrose content (up to 10 g / 100 g, Council Directive 2001/110/EC), attributed to the high sucrose content in acacia nectar.

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The analysis of pollens in kinds of honey primarily serves to determine the geographical origin of the honey samples, while also providing an opportunity to track changes in the plant biodiversity of an area. Salonen et al. (2009) drew conclusions based on pollen analysis about the multi-decadal changes in the natural vegetation and the cultivated plant crop area in different regions of Finland. There are only a few articles demonstrating the applicability of long-term melissopalinology for monitoring the alteration of biodiversity.

The aim of this article is to report the alteration of the floristics spectrum of plants in the last 20 y foraged by honeybees and in the identification of the most important plant sources for honey pollen. In this publication, a total of 19 samples of acacia honey harvested from the same area between 2000 and 2022 were examined to determine their pollen composition. Through monitoring, insights into the biodiversity of the vegetation within a 3 km radius of the apiary are gained.

2. Material and methods

2.1 Honey Samples

The examined acacia honey samples were harvested between 2000 and 2022 June. They originated from the apiary of Körmend (Western Hungary) with coordinates 47.02688723749258, 16.561321378377873, having more than 100 beehives 3 km radius of the monitoring area (Figure 1) encompasses various agricultural fields, urban zones, riverbanks, and highways. The individual yearly samples were stored at room temperature (20-21 °C), in a dark place, until commencement of analyses. The acacia honey samples collected over the years were received from the producers in April 2023, and the pollen analysis was conducted within one month of their arrival.

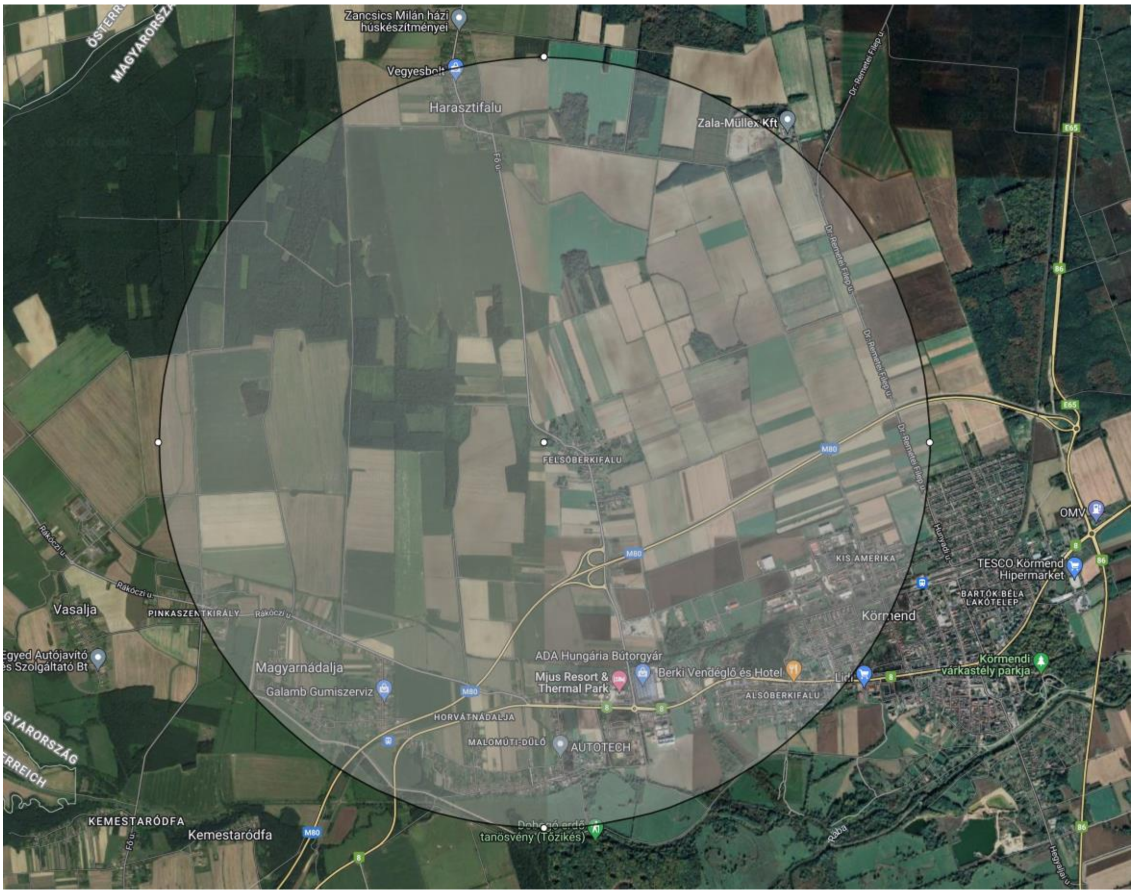

Figure 1: Monitoring area, 3 km radius of the apiary

2.2 Melissopalynological analysis

10 g of honey sample was measured from the homogenized sample into a 50 mL centrifuge tube, and 20 mL of distilled water was added. The honey solution was centrifuged for 10 min (RCF = 1,000 g), and after resting for 3-5 min, the supernatant was poured off. After adding 10 mL of distilled water, it was centrifuged again. After

the second centrifugation, the sample was allowed to stand again for 3-5 min, and then the supernatant was poured off. The remaining solution in the centrifuge tube (20-30 mL, depending on the honey sample) was applied with a micropipette to a glass slide prepared with lacquer felt, evenly distributed by drawing 20 x 20 mm cover plates around it. This provided the framework for the lacquer felt feather area. The sample applied to the slide was dried on a section drying hot plate at 40 °C (hand heat). Afterwards, the sample was stained with basic fuchsin dissolved in 20 % ethanol. The sample prepared in this way must be evaluated within five days. A permanent sample was also prepared from the pollen sample for later analysis. During this process, heated liquid glycerin gelatin was applied to the surface of the dried pollen preparation on the slide. The preparations were examined under a light microscope at 400 x magnification (Carl ZEISS Axio Imager 2). At least 500 pollens were identified in each sample (Behm et al., 1996).

3. Result and discussion

By conducting pollen analysis on homogeneous acacia honey samples from Körmend, obtained from the same producer and area for the years 2000-2022, the typical plant composition of the collection area was determined, as well as the number of nectar-giving plant species that define the botanical origin. During the pollen analysis, all flowering plants having pollens can be revealed (Salonen et al., 2009).

Since the nectar and pollen production of individual plant species varies, distinctions are made between plants that produce abundant nectar but little pollen (e.g., acacia, lavender, lime), plants that yield abundant nectar and a large amount of pollen, plants that produce little nectar but a lot of pollen (e.g., chestnut and forget-me-not), and plants that produce little of both nectar and pollen. The botanical origin can be excellently determined by examining the pollen content of honey, as its quantity and quality define the characteristics of the honey. In the case of varietal honey samples, it may happen that an insufficient amount of pollen from the specific plant is incorporated into the honey during the nectar production period. Therefore, in varietal honey samples, the relative frequency of pollens can vary significantly due to under- and over-represented pollens (Puusepp and Koff, 2014).

Overall, it can be stated that the examined 19 honey samples contain various pollen from forest trees, shrubs, fruit trees, weeds, field plants, ornamental plants, and agricultural crops. However, significant differences were observed between individual years in terms of both proportions and composition. In the examined honey samples, a total of 51 plant species were identified (Table 1). Among them, 40 species provide nectar or both nectar and pollen to bees, while 11 species provide only pollen to bees. Among the nectarless, insect-pollinated plants such as poppy, red poppy and tetterwort (*Papaveraceae)*, plantains (*Plantaginaceae*), and buttercups (*Ranunculaceae*). Among the nectarless, wind-pollinated plants such as pine (*Pinus spp.*) was present in each sample, and in numerous samples, sorrel family (*Rumex spp.*), meadow foxtail (*Alopecurus pratensis*), as well as sporadic occurrences of juniper (*Juniperus communis*) and hazelnut (*Corylus avellana*), and cypress family (*Cupressaceae*) were detectable. Furthermore, it is mentioned that traces of honeydew elements (fungal spores, hyphae, algae, wax particles) were found in all honey samples. These elements likely stem from protective pine trees near the beehives, shielding the bee colonies from adverse weather conditions (summer heatwaves, winter frosts, strong winds). Fungal structures and plant tissue elements are natural components originating from plants. However, none of the samples showed a significant presence of honeydew relative to nectar, with HDE/PG=0.05 (Min=0.01, Max=0.4), making it negligible for further evaluation. Only the honey from the year 2014, noticeably darker in colour compared to other samples, contained a small amount of honeydew, with HDE/PG=0.4 (Louveaux et al., 1978). This can be attributed to unfavourable weather conditions that led to a shortened acacia foraging period for bees in that particular year. In the majority of samples, organic debris contained other insect body parts. Among the inorganic materials, carbonaceous fragments, particularly soot, were most prevalent. On a positive note, no plastic fibre debris was observed in any of the samples.

In the microscopic images, budding yeast cells can be observed in the samples from the period between 2000 and 2009, appearing as clustered groups. Unidentified, cytoplasm-free, tiny pollen grains were found in only 2 samples, specifically in 2003 and 2005. The samples from the years 2000 to 2007 exhibited an exceptionally high average of 18.5 % (Min=5 %, Max=58 %) red clover (*Trifolium pratense*) pollen content, likely originating from the cultivated vegetation in the area. From the 2005 vintage onwards, Brassicaceae species were significantly detectable in the samples examined. Brassicaceae species have overrepresented pollen counts, which can distort the botanical composition of a given area. Like Trifolium pratense, these species are also water-demanding plants, mainly in the autumn and spring periods. A characteristic of the examined area is that the necessary higher relative humidity during the period of canola pollen binding is provided by the valley of the Rába River. Starting from the year 2008, grape pollen was detectable in the honey samples, albeit not in significant amounts, averaging 3 % (Min=0 %, Max=6 %).

Table 1: Other plant species identified in individual honey samples

Sample year	Other nectar producing plants	Other nectarless plants
2000	*Cornus sanguinea, Matricaria recutita, Leucanthemum vulgare*	*Pinus spp., Corylus avellana*
2003	*Rosaceae fruits* (Apple, Blackberry), *Salicaceae, Urtica dioica, Cornus sanguinea, Lamiaceae,*	*Pinus spp. Papaver rhoeas*
2004	*Cornus sanguinea, Rosaceae fruits* (Apple, Cherry, Pear), *Urtica dioica, Frangula alnus, Taraxacum officinale*	*Pinus spp., Papaver rhoeas*
2005	*Tilia cordata, Taraxacum officinale, Leucanthemum vulgare, Frangula alnus*	*Pinus spp., Chelidonium majus*
2006	*Knautia arvensis, Achillea millefolium, Rosaceae fruits* (Apple, Blackberry, Cherry), *Leucanthemum vulgare, Lamium album, Frangula alnus*	*Rumex spp., Pinus spp., Papaver orientalis, Chelidonium majus*
2007	*Lamiaceae (Teucrium chamaedrys, Lamium album), Wisteria sinensis, Rosaceae fruits* (Apple, Cherry)*, Clematis vitalba, Taraxacum officinale, Cornus sanguinea*	*Pinus spp., Papaver orientalis, Juniperus communis*
2008	*Rosaceae fruits* (Plum, Apple, Cherry) *Melilotus officinalis, Vitaceae, Clematis vitalba, Achillea millefolium, Matricaria recutita, Taraxacum officinale, Frangula alnus, Mercurialis perennis, Juglans regia*	*Pinus spp., Taxus baccata*
2009	*Vitaceae, Rosaceae fruits* (Blackberry, Strawberry), *Wisteria sinensis, Scrophulariaceae, Knautia arvensis, Clematis vitalba, Reseda lutea, Cornus sanguinea, Convolvulaceae, Anthriscus cerefolium, Salicaceae, Sambucus nigra, Frangula alnus*	*Rumex spp., Papaver rhoeas, Pinus spp., Alopecurus pratensis*
2010	*Vitaceae, Salicaceae, Sambucus nigra, Wisteria sinensis, Rosaceae fruits* (Apple, Cherry), *Juglans regia, Frangula alnus, Leucanthemum vulgare*	*Pinus spp., Papaver rhoeas, Alopecurus pratensis,*
2011	*Vitaceae, Loranthus europaeus, Lamium album, Frangula alnus, Taraxacum officinale, Anthriscus cerefolium*	*Papaver rhoeas, Poaceae, Pinus spp., Rumex spp.*
2012	*Vitaceae, Rosaceae fruits* (Cherry, Howthorn)*, Frangula alnus, Geraniaceae, Convolvulaceae, Melilotus officinalis, Taraxacum officinale, Juglans regia, Cornus sanguinea*	*Alopecurus pratensis, Pinus spp., Papaver rhoeas, Papaver orientalis*
2013	*Cornus sanguinea, Rosaceae fruits* (Howthorn, Cherry), *Scrophulariaceae, Lamiaceae, Trifolium repens, Knautia arvensis, Geraniaceae, Melilotus officinalis, Taraxacum officinale*	*Ranunculaceae, Rumex spp, Pinus spp.,*
2014	*Rosaceae fruits* (Plum, Apricot, Cherry, Howthorn)*, Cornus sanguinea, Lamium album, Juglans regia, Melilotus officinalis, Salicaceae, Frangula alnus, Viola arvensis, Vitaceae, Taraxacum officinale*	*Papaver rhoeas, Papaver orientalis, Rumex spp., Pinus spp., Plantaginaceae*
2015	*Melilotus officinalis, Achillea millefolium, Vitaceae, Cornus sanguinea, Salicaceae, Clematis vitalba, Lamiaceae, Taraxacum officinale, Juglans regia*	*Pinus spp., Papaver rhoeas, Ranunculaceae*
2016	*Viola arvensis, Persicaria spp., Rosaceae fruits* (Apple, Plum), *Frangula alnus, Matricaria recutita, Clematis vitalba, Daucus carota, Anthriscus cerefolium*	*Rumex spp., Pinus spp., Juniperus communis*
2017	*Pinus sativum, Frangula alnus, Rosaceae fruits* (Cherry, Apple, Howthorn)*, Taraxacum officinale, Juglans regia*	*Chelidonium majus, Pinus spp., Rumex spp., Papaver rhoeas*
2018	*Campanula spp., Leucanthemum vulgare, Rosaceae fruits* (Cherry, Blackberry), *Pinus sativum, Taraxacum officinale, Cornus sanguinea, Matricaria recutita, Anthriscus cerefolium*	*Chelidonium majus, Pinus spp., Rumex spp., Plantaginaceae*

Table 1: Other plant species identified in individual honey samples (continued)

2020	*Malva sylvestris, Trifolium repens., Salicaceae, Tilia cordata, Matricaria recutita, Achillea millefolium, Amorpha fruticosa, Persicaria spp., Capsella Bursa-pastoris, Viola arvensis, Rosaceae fruits* (Blackberry), *Leucanthemum vulgare, Loranthus europaeus*	*Rumex spp., Pinus spp., Cupressaceae*
2022	*Daucus carota, Capsella bursa-pastoris, Amorpha fruticosa, Wisteria sinensis, Vicia spp., Frangula alnus, Persicaria spp., Tilia cordata, Rosaceae fruits* (Strawberry, Howthorn), *Campanula spp., Leucanthemum vulgare, Vitaceae*	*Papaver rhoeas, Papaver orientalis, Pinus spp., Plantaginaceae*

In the honey from the year 2017, a remarkably high concentration (32 %) of forage peas (*Pisum sativum*) pollen was found. Based on the classification of the relative frequency of acacia pollens through the pollen analysis of the examined honey samples (Louveaux, et al., 1978), the acacia honey samples from the years 2004, 2010, 2016, and 2018 were of exceptionally good quality (Table 2). Using the absolute pollen density employed in the literature for determining geographical origin (Louvenaux et al., 1978), very low values were obtained, averaging 2,707 PG/10 g (Table 1), which also corroborates the acacia origin. The species that have appeared in recent years (*Daucus carota*, *Capsella Bursa-pastoris*) indicate the weediness of certain parts of the area, which can be explained by abandoned agricultural lands.

Table 2: Melissopalynological profile (Absolute pollen count, Pollen density and Botanical origin) characteristics per sample

Sample year	Absolute pollen count PG/10 g	Pollen density	Robinia pseudoacacia pollen (%)	Trifolium pratense pollen (%)	Brassicaceae pollen (%)	Other nectar producing plants (%)	Other nectarless plants (%)
2000	3,246	very low	28	59	0	13	3
2003	1,960	very low	33	9	12	46	4
2004	919	very low	73	8	8	11	1
2005	13,108	outlier	14	5	79	2	1
2006	2,573	very low	27	11	25	37	6
2007	2,695	very low	31	21	37	11	3
2008	1,225	very low	33	0	13	54	7
2009	3,524	low	27	0	27	46	6
2010	1,470	very low	51	0	22	27	5
2011	1,103	very low	37	0	46	17	2
2012	3,507	low	26	6	32	36	49
2013	2,328	very low	34	0	14	52	20
2014	2,144	very low	23	0	30	47	11
2015	1,960	very low	30	0	24	46	7
2016	1,838	very low	47	0	14	39	6
2017	2,205	very low	15	0	23	62	11
2018	1,228	very low	50	0	25	25	21
2020	1,899	very low	25	0	27	48	7
2022	2,511	very low	36	0	23	41	5

4. Conclusions

Based on the conducted research, pollen analysis of honey could potentially serve as a suitable method for monitoring the biodiversity of nectar and/or pollen-producing plants in the environment, as well as for observing long-term changes in species composition within a given agricultural and native area.

Throughout the examined 20 y period, the pollen components within the honey samples remained stable, and no observable decomposition of pollens was identified. The data spanning from 2000 to 2022 indicate a gradual increase in the number of species contributing pollen within the analyzed samples. Higher biodiversity enhances the stability and resilience of ecosystems, contributing to sustainability. However, further investigations are necessary to corroborate this observation.

The pollen analysis of acacia honey from the studied area reveals a consistent rise in biodiversity over time. Additional research is warranted to validate these observations.

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