UNIVERSITY OF SOPRON
Faculty of Forestry

Doctoral (PhD) theses

ASSESSMENT OF AGROFORESTRY AREAS USING BEE BIOMONITORING

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1. Topicality of the subject

Agroforestry systems are receiving increasing attention worldwide as effective solutions for adapting to climate change and mitigating its negative impacts. The integrated cultivation of woody plants, agricultural crops, and livestock provides a wide range of ecosystem services: it improves soil water balance, reduces erosion, enhances carbon sequestration, and increases biodiversity. In a landscape-scale approach, agroforestry plays a key role in developing sustainable land use, as it combines agricultural production with ecological functions, thereby contributing to the environmental and economic stability of rural areas. Structurally diverse habitats offer favorable conditions for pollinators by providing more stable nectar and pollen sources, which helps to maintain the health of honeybee colonies and the sustainability of honey production.

One of the modern and cost-effective methods for monitoring biodiversity is bee biomonitoring, which is based on the analysis of hive products collected by honeybees (*Apis mellifera*) including pollen, honey, propolis, and wax. As bioindicators, bees collect representative samples over large areas, enabling an integrated assessment of environmental conditions. The examination of pollen samples provides detailed information on vegetation composition, flowering dynamics, and the occurrence of both invasive and protected plant species.

Beekeeping today faces serious challenges due to climate change. Extreme weather events such as droughts, mild winters, and late frosts directly affect nectar and pollen yields. In Hungary, the production of acacia and rapeseed honey has become particularly unpredictable, raising both economic and ecological concerns.

The aim of our research is to contribute to understanding the ecological and economic challenges affecting beekeeping, as well as the interactions between climate change and agroforestry systems, through the complex analysis of pollen loads and monofloral honeys collected by bees.

2. Research Objectives

1. Investigation of Honeybee Foraging Preferences in an Agroforestry Environment

   Our aim is to compare the plant selection preferences of honeybees in two agroforestry sites (Harka and Bajna) based on pollen loads and monofloral honeys collected in 2022–2023, using microscopic pollen analysis. We investigate whether honeybees prefer the same or different plant species during nectar and pollen collection, and which type of sample shows greater species richness, which may also provide insights into the adaptive capacity of the bees.

2. Characterization and Standardization of Monofloral Honeys

   Our aim is to conduct a comparative analysis of economically important acacia and rapeseed honeys based on pollen analysis and gas chromatographic examination of volatile organic compounds (VOCs). The results will contribute to the development of a reference database that enables more accurate identification and characterization of samples originating from agroforestry areas.

3. Evaluation of Honey Quality Using a Multi-Analytical Approach

   Our aim is to conduct a comprehensive quality assessment and classification of commercial acacia and rapeseed honeys using a multi-analytical approach. In this study, we intend to identify the parameters most suitable for reliably distinguishing the two honey types by analyzing physicochemical properties, elemental composition, and melissopalynological characteristics. Our results may contribute to improving the accuracy of honey authentication and to refining the current EU regulations—which are largely based on pollen content, namely Directive 2001/110/EC on honey and its amendment 2014/63/EU—since the present system can produce biased results for plant species with underrepresented pollen counts.

4. Long-Term Biodiversity Monitoring Using Pollen Analysis

   Our aim is to monitor changes in the vegetation of a given area over a 20-year period using microscopic pollen analysis. Comparing the pollen composition of annual samples provides an opportunity to explore the long-term dynamics of the ecosystem and cultivated crops.

3. Materials and methods

Description of the Research Sites

In our study, we investigated several agroforestry areas with diverse landscape and ecological characteristics. The sampling locations were situated in the regions of Harka, Bajna, Sopron, Körmend, Nagycenk, and Barbacs. The research was based on data from the COPERNICUS program, which allowed us to determine the spatial arrangement and mosaic structure of each site.

Analytical Methods

- Melissopalynological Analysis (Light Microscope, 400× Magnification)
    - For the analysis of pollen loads, sample preparation was carried out according to the method of Barth et al. (2010): 2 g of each pollen load was purified by ethanol centrifugation, then suspended in a water–glycerin solution and centrifuged again. A sample from the resulting suspension was placed on microscope slides, which were covered with a glycerin–gelatin mounting medium after drying for microscopic examination.
    - For honey samples, analysis was performed following the preparation of homogenized samples, based on the methods of Rex Sawyer (2010) and Pendleton (2006), as well as the Hungarian standard MSZ 6950-3. Ten grams of honey were diluted with distilled water and purified by two rounds of centrifugation. A sample from the resulting suspension was placed on microscope slides and covered with a glycerin–gelatin–fuchsin mounting medium for microscopic observation.

- Determination of Physicochemical Parameters of Honey Samples
    - The moisture content and Brix value of the honey samples were determined using a refractometric method according to the Hungarian standard MSZ 6943/1-79, employing an ATC-3 portable refractometer (China).
    - pH and acidity measurements were conducted in accordance with the Hungarian standard MSZ 6943/3–80.
    - Electrical conductivity of the honey samples was measured (Radelkis OK-114; Radelkis OK-0907P electrode, Hungary) on a 20% (w/w) honey solution at 20 °C (Bogdanov, 1997; Czipa et al., 2019; Pospiech et al., 2021).
    - Diastase activity was determined according to the Hungarian standard MSZ 6943/6–81.
    - HMF (hydroxymethylfurfural) content was measured spectrophotometrically according to the Hungarian standard MSZ 6943/5–1989.

- Chromatographic Analysis of Honey Samples
    - The sugar composition of the honey samples (fructose, glucose, and sucrose) was determined using high-performance liquid chromatography (HPLC) with a Shimadzu LC-20 system and RID detector.
    - A The volatile organic compounds (VOCs) in the honey samples were analyzed using solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC-MS). Analyses were performed with a SHIMADZU GCMS TQ8040 system equipped with a medium-polarity TG-5MS capillary column.

- Determination of the Elemental Composition of Honey Samples
    - Approximately 0.4 g of each homogenized sample was weighed into Teflon-coated digestion vessels. Microwave digestion was performed using HNO_3 and H_2O_2, and after filtration, the resulting solutions were analyzed for elemental content using ICP-OES.

Statistical Methods Applied

- Testing the normality of variable (indicator) distributions in honey samples and identifying outliers,
- Confidence intervals for normally distributed variables
- Comparison of means between different honey types using Student's t-test
- Correlation analysis by honey type
- Principal Component Analysis (PCA)
- Determination of Wilks' lambda values among honey groups in the principal component coordinate system.

For the implementation of the evaluation strategy, the software environment included Microsoft Excel, the Chemometrics Add-In, the StatsKingdom online platform, and the Scilab FACT 1.4.2 chemometrics extension. The PCA-based clustering of acacia, rapeseed, and agroforestry honey samples was analyzed using fuzzy clustering (Borosy et al., 2001) in Scilab, while meteorological and pollen analytical data were processed and visualized using Python.

4. Research outcomes, conclusions and theses

Our research examined the relationship between agroforestry areas and apicultural products, highlighting the role of these systems in maintaining biodiversity, pollinator health, and honey quality. Using microscopic pollen analysis and VOC profiling, we studied the botanical and chemical composition of acacia, rapeseed, and agroforestry honeys, as well as pollen loads. Based on our results, the composition of honeys and pollens varied significantly by site, year, and product type; the wetter year (2023) resulted in greater plant diversity.

Fuzzy clustering of the VOC analysis revealed a distinct volatile profile for acacia honeys, while rapeseed honeys showed partially overlapping profiles, indicating the chemical heterogeneity and uniqueness of agroforestry honeys. Evaluation of physicochemical, trace element, and pollen data using PCA and t-tests showed that acacia and rapeseed honeys can be clearly distinguished, with the main differences appearing in sugar composition (glucose, fructose/glucose ratio) and electrical conductivity.

The current EU classification for acacia honey proved to be overly strict; therefore, the inclusion of additional indicators is recommended. Examining a 20-year dataset, we found that the pollen composition of acacia honeys remained stable, while biodiversity showed an increasing trend, suggesting that honey can serve as a reliable tool for long-term ecological monitoring.

THESIS 1

In the case of rapeseed and acacia honeys, the greater biodiversity of agroforestry areas is indicated by the higher values of the "rare pollen" frequency class. In agroforestry acacia honey samples, this value is 8-39% (average 18%), while in monofloral honeys, it is 2-23% (average 8.65%). For rapeseed honeys, in agroforestry samples, it is 7-24% (average 12.75%) compared to the 1-5% (average 2.75%) proportion in monofloral honeys.THESES

THESIS 2

The volatile organic compound (VOC) profile of honeys originating from agroforestry systems shows significantly greater chemical diversity than that of monofloral honeys from conventional agricultural areas. Based on a 50% occurrence threshold, 33 VOC components were detectable in acacia honeys collected from agroforestry systems, whereas only 13 were found in conventional monofloral acacia honeys. A similar trend is observed for rapeseed honeys: samples of agroforestry origin are characterized by 34 components, while conventional rapeseed honeys have 22.

THESIS 3

SPME-GC-MS analytical testing based on volatile organic compounds (VOCs) proves to be a suitable method for identifying the origin of honeys harvested from agroforestry systems following rapeseed and acacia blossoming, which is comparable in its effectiveness to traditional pollen analysis used as a reference. During the processing of the results from the 18 examined agroforestry honey samples using multivariate data analysis (Fuzzy method), pollen analysis distinguished the samples from the monofloral honeys (acacia and rapeseed) serving as a reference database in 7 cases, whereas VOC analysis did so in 10 cases, which indicates the greater discriminatory power of the VOC-based method.

THESIS 4

The analysis of a large number of Hungarian acacia honey samples has shown that monofloral honey classification based solely on pollen analysis is not reliable in all cases. Instead of pollen analysis, the combined assessment of the following chemical parameters of the examined samples – fructose/glucose ratio, glucose content, conductivity, acidity, and magnesium content – is suitable for determining the monofloral character of acacia honey samples.

THESIS 5

Based on the microscopic pollen analysis of acacia honey samples harvested between 2000 and 2022 from the Körmend agroforestry area, it can be established that certain Fabaceae species cultivated in agricultural areas – particularly crimson clover (Trifolium incarnatum) and fodder pea (Pisum sativum) – serve as a significant floral source for honey bees (Apis mellifera) during the acacia flowering period. The attractiveness of these species significantly influences the pollen spectrum of the acacia honeys, which impacts the classification of the honeys according to botanical origin, as well as their quality assessment.

5. Publications on the subject of the thesis

Publications in Q1-Q4 Journals

DOMINKÓ, E. – NÉMETH, ZS. I. – RÉTFALVI, T. (2024): Classification of acacia, rape and multifloral Hungarian honey types. Heliyon. E30498. DOI:https://doi.org/10.1016/j.heliyon.2024.e30498 (Q1)

DOMINKÓ, E. – KOVÁCS, Z. – RÉTFALVI, T. (2023): The Role of Pollen Analysis in the Sustainable Development. *Chemical Engineering Transactions*. 107. 673-678. DOI: 10.3303/CET23107113 (Q3)

Conference Paper – Full Text, Peer-Reviewed, Foreign Language

DOMINKÓ, E. – RÉTFALVI-SZABÓ, P. – KOVÁCS, Z. – LAKATOS, F. – RÉTFALVI T. (2020): Investigation of VOC components of honey. Proceedings of the International Conference on Sustainable Economy and Agriculture. 14th november 2019, Kaposvár Hungary. pp. 85-90

Peer-Reviewed Book Chapter

DOMINKÓ E. – RÉTFALVI T. (2023): Agrárerdészeti rendszerekből származó mézminták pollenanalízise. Az Erdőmérnöki Kar Tudományos kiadványa (szerk: Czimber Kornél) Soproni Egyetem Kiadó. 2023. 282 p. pp. 74-79.

DOMINKÓ E. – RÉTFALVI T. (2022): Mézek aromakomponenseinek összehasonlító vizsgálata az Erdőmérnöki Kar Tudományos Kiadványa (szerk: Czimber K., Heil B.) Soproni Egyetem Kiadó. 2022. 297 p. pp. 80-84.

DOMINKÓ, E. – RÉTFALVI, T. (2021): Hazai mézek kémiai analitikai vizsgálata. Soproni Egyetem Kiadó. pp. 439-445.

DOMINKÓ, E. – RÉTFALVI, T. (2021): Hazai mézek gázkromatográfiás vizsgálata. Soproni Egyetem Kiadó. pp. 446-452.

Conference Abstract

DOMINKÓ, E. – KOVÁCS, Z. – RÉTFALVI, T. (2024): A GreenBee Projekt referencia területeiről származó pollenek vizsgálati eredményei. Erdészeti Tudományos Konferencia. Sopron, Hungary. (Abstrakt/ Kivonat) 75 p. p. 51.

Other – Full Text, Non-Peer-Reviewed

DOMINKÓ, E. – KOVÁCS, Z. – RÉTFALVI, T. (2023): Pollen, több mint allergia! Website post on the official webpage of the Faculty of Forestry, University of Sopron.

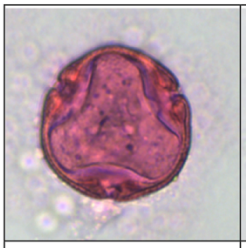
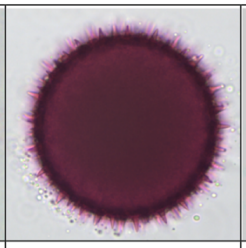
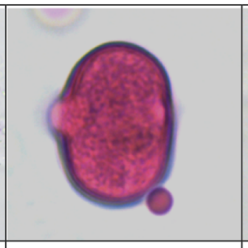
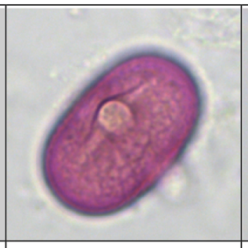
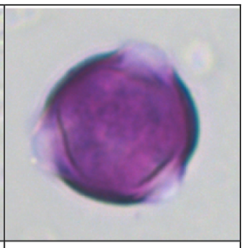
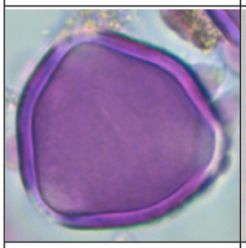
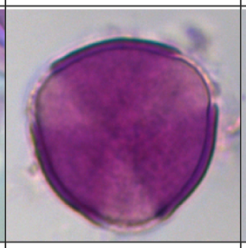
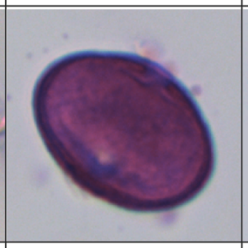
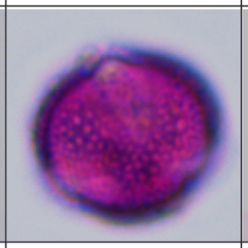
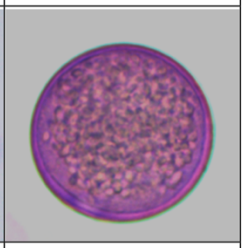
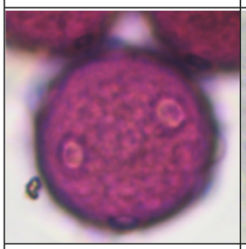
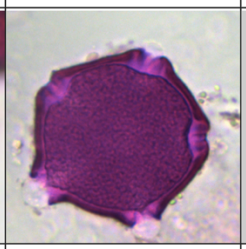
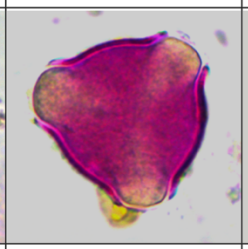
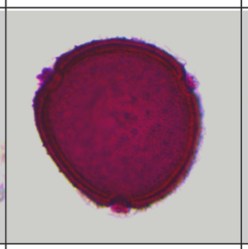
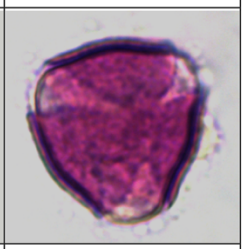
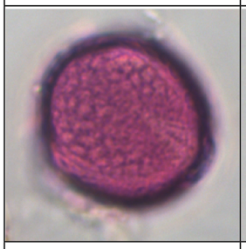
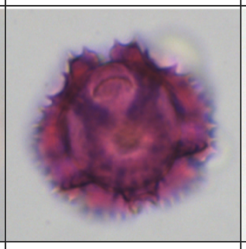
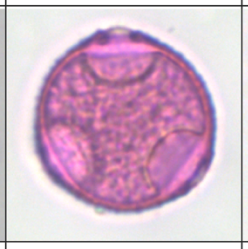
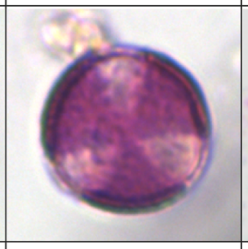
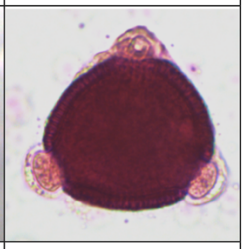
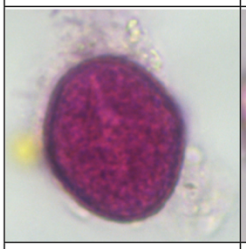
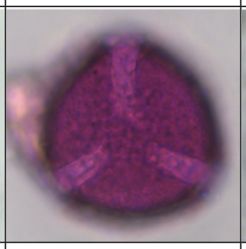
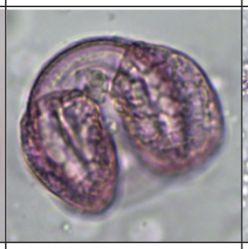
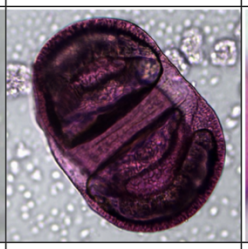
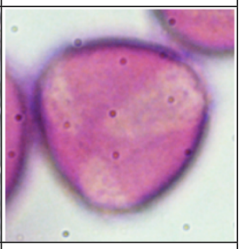
Conference and Presentation Materials

DOMINKÓ, E. – KOVÁCS, Z. – RÉTFALVI, T. (2023): Pollen, több mint allergia! Pecha Kucha presentation. Sopron, Hungary.

DOMINKÓ, E. – RÉTFALVI, T. (2022): Mézek aromakomponenseinek összehasonlító vizsgálata. Vegyészkonferencia, Eszterházy Károly Katolikus Egyetem, Eger, Hungary. (poster)

DOMINKÓ, E. (2022): Pollenvizsgálati eredmények mézmátrixban. Erdészeti Tudományos Konferencia. Sopron, Magyarország (oral presentation).

DOMINKÓ, E. – RÉTFALVI-SZABÓ, P. – KOVÁCS, Z. – LAKATOS, F. – RÉTFALVI T. (2019): Investigation of VOC components of honey samples. Proceedings of the International Conference on Sustainable Economy and Agriculture. 14th november 2019, Kaposvár Hungary (oral presentation).

				
Tilia platyphyllos nagylevelű hárs 31-35 µm	*Malva sylvestris* erdei mályva 84-123 µm	*Melilotus officinalis* orvosi somkóró 22-28 µm	*Pisum sativum* takarmányborsó 32-48 µm	*Sambucus nigra* fekete bodza 17-20 µm
				
Wisteria sinensis lilaakác 30 µm	*Lamium purpureum* piros árvacsalán 26-34 µm	*Anthriscus cerefolium* zamatos turbolya 21-25 µm	*Salix spp.* fűz fajok 17-23 µm	*Alopecurus pratensis* réti ecsetpázsit 26-31 µm
				
Plantago lanceolata lándzás útifű 21-28 µm	*Viola arvensis* mezei árvácska 51-100 µm	*Prunus cerasus var.* meggy 36-40 µm	*Knautia arvensis* mezei varfű 95-110 µm	*Rubus spp.* szeder fajok 28-37 µm
				
Trifolium incarnatum bíborhere 30–40 µm	*Taraxacum officinale* pongyola pitypang 28 µm	*Campanula spp.* harangvirág fajok 29-36 µm	*Capsella bursa-* pastoris pásztortáska – 19 µm	*Geranium spp.* gólyaorr fajok 67-87 µm
				
Trifolium pratense réti here 35-42 µm	*Cornus sanguinea* veresgyűrű som 58 µm	*Pinus nigra* fekete fenyő 55-77 µm	*Picea abies* lucfenyő >100 µm	*Papaver somniferum* kerti mák 28-32 µm