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POLLEN DIVERSITY IN HONEY OF THE CZECH REPUBLIC IN THE 2019 SEASON

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ABSTRACT

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Honeybees are important pollinators. As a side product of pollination, honeybees produce honey, as a natural sweetener. The source of honey depends on the hive location. In specific conditions honeybees produce monofloral honey, but more common are polyfloral kinds of honey. In this study honey from the Czech Republic in the 2019 season was evaluated by melissopalynology analysis. The common botanical taxa in the Czech Republic were determined and season impact to pollen taxa was compared for dominant pollen taxa. The taxonomic distribution of pollen in Czech honey was stable during the year. The average number of species was 11.52 taxa per sample. The dominant pollen source in Czech honey was the *Brassicaceae* family. The high pollen content in honey was confirmed also in the *Rosacea* family (fruit tree), *Ubelliferacae* family and *Myosotis* genus. During the year the pollen taxa were equally distributed in honey. Seasonal effects were confirmed only in *Salix* genus, *Ubelliferacae* family and *Phacelia* genus. Seasonal effects correspond with the blooming season and honeybee handling in the hive was also confirmed. High variability during the season and hive location was confirmed for other taxa.

Keywords: melissopalynology; botany taxa; pollen; biodiversity; adulteration

INTRODUCTION

The Czech Republic is one of the Central European countries. Its geographical position roughly in the center of the European continent means that the flora in this country includes plant species from the cold north and warm south as well as the oceanic west and continental east. The country is covered by a heterogeneous mosaic of cultural landscapes with arable fields, deciduous, mixed and coniferous forests, meadows, pastures, and human settlements. The dominant type of natural vegetation is a forest. Natural treeless vegetation includes alpine and subalpine grasslands, steep rocky slopes, steppe, peat bogs, and natural water bodies (Kaplan, 2012). The flora includes 148 families of vascular plants, 925 genera, 3754 species and subspecies, and 618 hybrids. Genera with 30 or more species include Taraxacum (221 species), Rubus (127), Carex (85), Hieracium (59), Pilosella (59), Veronica (35), and Trifolium (34), four of which include agamospermous species, which accounts for the high diversity. Families richest in species are the Asteraceae (666 species), Rosaceae (315), Poaceae (273), Fabaceae (171), Brassicaceae (148), Cyperaceae (127), Lamiaceae (112), Caryophyllaceae (108), and Apiaceae or Umbelliferae (99) (Danihelka, 2013). Due to the human factor activity, the landscape changes and some plant species gradually disappear (Grulich, 2012), while other non-native plants are introduced into the Czech ecosystem (**Pyšek et al., 2012**). The list of botanical species is extensive. In addition to the diversity and stability of the landscape, some taxa also participate in the honey collection of bees in the Czech Republic. The share of individual plants in the honey collection varies. It all depends on the amount of nectar and pollen produced, which is reflected in the different attractiveness of botanical species for bees. The pollen grains present in honey can be used to determine the botanical origin of it (Von Der Ohe et al., 2004).

Table 1 N	umber of pollen	grains in	selected	unifloral l	honey
(Demiano	owicz, 1964).				

Type of unifloral honey	The average number of					
	pollen grains / 10 g of					
	honey,					
Myosotis silvatica	147,456,000					
Brassica napus	72,000					
Taraxacum officinale	18,000					
Malus domestica	18,000					
Robinia pseudoacacia	1,125					
Phacelia tanacaetifolia	72,000					
Tilia sp.	2,250					

<u>Iaxon</u> Brassica sp	29.22	<u> </u>	20.36	<u> </u>	1 44	<u>L*a*b*</u> 94 30:-5 09:21 62	
Corvlus sp	27.62	2.04	19.31	1.75	1.43	98.67:-5.70:14.66	
Artemisia sp.	23.64	1.95	16.26	0.93	1.45	97.27;-3.96;11.18	
Alnus sp.	38.90	19.31	26.86	13.42	1.45	97.04;-5.20;16.05	
Fruit tree	42.47	8.68	24.17	4.72	1.76	97.61;-2.09;6.21	
Robinia sp.	33.58	2.93	21.42	3.36	1.57	98.02;-3.12;9.65	
Rubus sp.	23.64	2.51	14.89	1.86	1.59	97.40;-3.96;11.35	
Salix, Salicaceae	18.99	0.8	12.78	0.82	1.49	96.73;-5.02;16.07	
Bellis sp.	31.53	1.05	19.66	1.35	1.60	91.94;-4.37;18.91	
Acer sp.	41.66	2.06	22.52	2.41	1.85	93.76;-1.27;16.43	
Helianthus sp.	33.05	4.03	26.33	3.56	1.26	96.38;-3.59;17.32	
Fagus sp.	33.96	6.15	23.43	4.22	1.45	99.27;-3.24;6.37	
Trifolium sp.	25.73	4.47	15.52	2.85	1.66	96.65;-3.58;12.96	
Tilia sp.	20.1	3.95	14.18	2.87	1.42	96.35;-2.72;10.78	
Phacelia sp.	20.76	1.58	14.38	1.23	1.44	95.58;-4.22;13.89	
Rhamnus sp.	59.46	5.29	24.15	2.26	2.46	98.30;-3.44;20.23	
Umbelliferae	23.67	2.5	13.75	1.34	1.72	96.96;-3.64;11.75	
Achillea sp.	33.49	6.3	20.11	2.15	1.67	97.43;-3.22;11.35	
Vicia sp.	44.1	3.86	30.29	5.3	1.46	91.74;-2.39;24.78	
Taraxacum sp.	34.71	2.05	21.95	2.68	1.58	94.28;-6.39;39.21	
Myosotis sp.	14.56	2.86	7.29	1.3	2.00	95.07;-3.13;10.04	

Table 2 Referee values for morphological and spectral characteristics

However, when determining the botanical origin, it is necessary to consider the unlike the production of pollen by botanical taxa, which was experimentally verified in the study by Deamianowics 1964 (**Table 1**).

At present, the pollen profile typical for Czech honey has not been described. Such data can be used not only to expand knowledge but can also play a crucial role in preventing honey adulteration. The origin of honey can be proven under certain conditions based on the pollen profile that represents the area where the honey comes from (Aronne and de Micco, 2010; Soares et al., 2017). Some countries have their pollen profile of honey described. Monofloral honey is the ones characterized most commonly (Persano Oddo and Piro, 2004; Oddo et al., 1995; Persano Oddo et al., 2004; Feás et al., 2010; Karabagias et al., 2020). Fewer studies have focused on the pollen profile of polyfloral honey (Kuś et al., 2018; Čeksteryte, Kurtinaitiene, and Balžekas, 2013; Kale Sniderman et al., 2018; Puusepp and Koff, 2014; Jones and Bryant, 2014). For the characterization of monofloral honey, in particular, the number of pollen grains of the species as well as the amount of accompanying pollen grains must be taken into account.

This study aims to bring new knowledge about the pollen profile of Czech honey in 2019.

Scientific hypothesis

The pollen profile of honey is closely dependent on the area of collection of nectariferous and nectarless plants around the hive. Pollen profile variation during the year was verified in this study.

MATERIAL AND METHODOLOGY Sampling Collection

The experimental material was collected from individual colonies of western honeybee *Apis mellifera carnica*. One or two sealed honeycombs from each colony were extracted using a common hand extractor. All samples were from the 2019 season. The samples were collected from May to August and classified into four groups depending on the month of their collection. The 163 samples were evaluated from 130 different areas of the Czech Republic with various geographical profiles and botanical origins. Pollen profile in posterior months is influenced by the natural handling of honey in the hive. The honey handling can cause temporal and also positional shifts according to beekeeping practice **(Vorwohl, 1972)**.

Pollen Analysis

Honey samples were prepared following the guidelines of the International Commission of Bee Botany published by von der Ohe (Von Der Ohe et al., 2004). Glycerol-gelatine preparations were made in duplicate for each honey. The pollen spectrum was evaluated by Nikon Eclipse Ci-L (Nikon, JPN). The slide was automatically scanned by the motorized stage with the focus motor of Proscan III (Prior, USA). Images were captured in stag files using DFK 23U274 camera (Imaging Source, GER). The position was chosen randomly. The magnifications used were 100x and 400x.

The pollen spectrum was classified according to Stawiarich (Stawiarz and Wróblewska, 2010) into four groups >45% dominant, 16 - 45% secondary, 3 - 15%

important minor and <3% minor pollen. Pollen discrimination was performed according to Moar (Moar, 1985). At least 300 pollen grains were counted in each preparation, where pollens were identified according to melissopalynology atlas (El-Labban, 2020) to the most possible exact taxon – species, genus, type of structure or family in classes >3%. Pollen not clearly identified by the evaluator was evaluated by morphometric and spectral characteristics obtained from image analysis. Referee values are shown in Table 2.

Statistical analysis

The data were processed statistically using the 2014.5.03 XLSTAT software (Addinsoft, USA). The normality test confirmed the not normal distribution of the data. A nonparametric Kruskal-Wallis test was used to compare the pollen profile in months and the diversity of pollen taxa during the year.

RESULTS AND DISCUSSION

Honey samples were collected during the 2019 beekeeping season, specifically from 18 May to 16 August 2019. The period was selected to include both spring and summer honey phases. Such a long period of the collection of samples enables covering botanical taxa of nectar-producing and pollen-producing plants involved in honey production in the Czech Republic. 163 honey samples were taken. Of these, 30 honey samples could be characterized as monofloral concerning their pollen spectrum. These were namely (*Brassica sp. 8; Prunus sp., Pyrus sp. 18; Tilia sp.* 4). It is generally stated that if there is more than 45% of pollen grains of one species in certain honey, the honey can be described as single-species or monofloral. However, this rule does not apply to all botanical taxa. Some plants differ in their pollen-producing capacity, both in the high content

of pollen grains and, conversely, due to the low content of pollen grains (**Demianowicz**, 1964). The rule that several botanical species contribute to the composition of nectar and pollen content even in monofloral honey also applies (**Louveaux**, **Maurizio**, and **Vorwohl**, 1970). Of the analyzed samples, 133 kinds of honey were polyfloral. The representation of individual taxa in Czech honey is summarized in **Figure 1** and these taxa are divided into four groups, namely <3% minor pollen, 3 - 15% important minor 16 - 45% secondary and >45% dominant.

The wide pollen spectrum in Czech honey is characteristic of Mediterranean areas with a high degree of urbanization and agricultural activity. Even about the large sown areas of crops in the Czech Republic, Czech honey retains in most cases the character of polyfloral honey. Differences in diversity by hive location were described in a French study **(Odoux et al., 2012)**. Here, the authors confirmed a reduction in the diversity of pollen taxa in agricultural areas. For the Czech Republic, however, there has been no similar study yet that would allow a comparison of the change in the number of pollen taxa in agricultural areas. The results of the study showed an average of 11.63 pollen taxa in pollen with a proportion of >3% occurrence of pollen grains in honey **(Figure 2)**.

Another monitored parameter was the differences in the number of pollen taxa throughout the honey season. Taxa with a frequency of presence greater than 3% pollen grains were observed (Figure 2). Figure 2 shows the number of pollen taxa during the year per sample. A shorter distance between curve points means a shorter period between sampling in a given period. The time interval between sampling at the beginning and end of the season is longer. Both the climatic conditions and the source of nectar at the given habitats affect the time interval.



Figure 1 Absolute frequency of major pollen taxa in Czech honey in 2019.



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Figure 2 Taxa diversity in 2019.

For the Czech Republic, no statistically significant differences were recorded between the date of honey extraction and the diversity of pollen taxa (p > 0.05). This result is also confirmed by Figure 2 which does not confirm the change in individual months. The differences in the number of pollen taxa correspond to specific habitats, not to the time of the year. The average diversity in the spring was 12 taxa in May and 11.62 taxa in June. In summer it was 10.83 taxa in July and 11.63 taxa in August. The result is in agreement with the study (Avni et al., 2014) where, using chemical analysis, the authors showed that the amount of pollen in the pollen collection does not differ during the year, but is dependent on the habitat. Primarily in the spring period, pollen is responsible for the rapid development of bee colonies (Odoux et al., 2012) and subsequently contributes to honey yields. In the conditions of the Czech Republic, spring pollen includes primarily pollen of fruit trees. Fruit tree pollen is considered a very good source of protein for bee colonies (Roulston and Cane, 2000). Due to its nutritional importance, fruit tree pollen plays an important role in bee nutrition and is associated with a high preference for bees. Even concerning lower pollenproducing capacity (Table 1) than in Brassica sp., the pollen of fruit trees was represented in honey on average in the amount of 8.82%, in most cases, it was an important minor (Figure 1). Differences between the compared months were not confirmed, but the proportion of pollen grains of fruit trees in later months, as well as other pollen sources, confirm the pollen cycle in honey within a year (Figure 3 and Figure 4). The occurrence of pollen of spring botanical species in later months is mainly due to the growth of the brood and the transport of pollen both on bees as well

as by bees to their honeycombs. The influence of the pollen profile on the distance from the brood, but also the humidity in the hives was confirmed by Spanish authors (Da Fernandez and Ortiz, 1994). Fruit trees include several genera and even more cultivars, but concerning their close relationship, the morphology of the pollen grain is similar, although there are differences between the pollen grains, especially in the color of the pollen (Pospiech et al., 2019). For melissopalynological purposes, they are often taken as one group (Stawiarz and Wróblewska, 2010). The fact that the honeybees' visits to flowers are not influenced by an exclusive species preference also makes it difficult to determine the exact species. On the other hand, the species non-specificity of the honeybee is used in orchards, where the bee is a significant pollinator (Cunningham et al., 2016). Single-species honey of fruit trees is not widespread. Their occurrence has been described, for example, in Bulgaria. Due to their sensory closeness, they may be confused with other spring honey, primarily with black locust honey (Atanassova, Yurukova, and Lazarova, 2012). A high proportion of pollen from *Brassicaceae* and fruit trees was also found in Polish polyfloral honey, see Table 3 (Stawiarz and Wróblewska, 2010).

Other important sources of pollen in spring honey include pollen of the *Brassicaceae* family (especially *Brassica sp.*), dandelion, hazel, and black locust (Figure 3). High *Brassica sp.* pollen content is recorded primarily in honeybee colonies near the agricultural areas with these plants (Danner et al., 2016). In these areas, honey can also reach the character of monofloral honey.



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Figure 3 Average occurrence of pollen of spring botanical taxa.



Figure 4 Average occurrence of pollen of summer botanical taxa.

Due to the high pollen-producing capacity, honey with a rapeseed pollen content of more than 80% can be considered monofloral rapeseed honey (El-Labban, 2020). Of the honey analyzed in our study, 8 honey samples would meet this definition. However, high content of rapeseed pollen grains was also recorded in polyfloral honey, with more than 16% of pollen grains in 106 honey samples.

The high content of pollen grains of *Brassica sp.* is mainly due to its pollen-producing capacity (**Table 1**). It was experimentally verified that it belongs among the most pollen-producing plants involved in spring honey collection (**Demianowicz, 1964**). We found the average proportion of rapeseed pollen at 35.43%, in most cases it was the secondary pollen (**Figure 1**). The high proportion of rapeseed pollen was also confirmed in Polish and Estonian honey (**Stawiarz and Wróblewska, 2010; Puusepp and Koff, 2014**).

Salicaceae pollen is considered to be an important source of protein needed for honeybee colony development, although willow also provides some nectar. Honeybees mainly search for male flowers for their source of pollen grains (Dötterl et al., 2014). The average proportion of Salicaceae pollen was 4.2% and in most cases, it was minor pollen. Our results showed statistically significant differences in the amount of Salicaceae pollen grains in May-June honey, compared to July-August honey (Figure 3). The august increase shows also the temporal shift of pollen caused by handling in the hive as confirmed previously by (Da Fernandez and Ortiz, 1994) and (Vorwohl, 1972) for Myosotis pollen. Although monofloral Salicaceae honey is not described in the Czech Republic, monofloral Salicaceae honey can be found in Croatia, Spain, Lithuania, and New Zealand (Jerković and Marijanović, 2010).

	Czech Republic*			Portugal (Sousa et al., 2014; Feas et al., 2012)			Poland (Stawiarz and Wróblewska, 2010)			Estonia (Puusepp and Koff, 2014)		
Taxon	x	Min.	Max.	x	Min.	Max.	x	Min.	Max.	x	Min.	Max.
Robinia sp.	2.43	0.17	16.01	16.00	13.00	22.00	28.13	3.00	27.70	8.50	8.50	8.50
Fruit tree	8.88	0.3	65.29	23.36	1.00	80.00	30.40	4.00	21.10	14.22	0.80	56.90
Rhubus sp.	1.38	0.17	12.25	4.00	2.10	5.60	-	-	-	4.91	0.60	16.70
Trifolium sp.	3.47	0.15	33.62	13.30	4.40	45.60	18.10	-	-	11.90	0.40	71.40
Brassica sp.	35.43	0.46	94.12	12.00	12.00	12.00	33.26	16.2	59.60	9.81	2.00	31.30
Taraxacum sp.	2.23	0.15	16.41	-	-	-	40.20	40.2	40.20	4.23	0.30	13.60
Phacelia sp.	8.68	0.17	40.8	-	-	-	26.73	3.00	18.70	12.15	4.80	19.50
Salixaceae	4.02	0.19	19.29	-	-	-	30.57	16.3	44.10	11.30	2.73	29.00
Tilia sp.	3.52	0.17	25.38	-	-	-	-	-	-	44.74	1.30	79.03
Umbelliferae	10.03	0.29	65.23	-	-	-	-	-	-	15.80	0.50	82.80

Table 3 Percentage of pollen in polyfloral honeys of selected botanical taxa.

Note: * honeys with a pollen content higher than 0 are included.

Robinia pseudoacacia pollen was confirmed in honey in all monitored months. Its average representation was 2.43%. In most cases, it had a minor representation, which corresponds to the findings by other authors (Stawiarz and Wróblewska, 2010; Čeksteryte, Kurtinaitiene, and Balžekas, 2013). In June, the content of *Robinia pseudoacacia* pollen was the highest and one sample contained 16.01% of this pollen. Although some authors state that >15% of *Robinia pseudoacacia* pollen indicates monofloral honey (Oddo et al., 1995), more authors are inclined to the 20% limit (El-Labban, 2020). The reason for the different minimum limit of pollen grains in singlespecies black locust honey is mainly the low pollenproducing capacity (Table 1) and the high nectar-producing capacity of black locust.

Monofloral dandelion honey is also characterized by a low content of pollen grains, which is usually in the range of 5 – 15% in monofloral honey as well (Jerković et al., 2015). In the pollen profile of these honey, dandelion pollen is often lower than the associated species, such as Salix or Cruciferae (Persano Oddo and Piro, 2004). The average proportion of dandelion pollen was 3.62% and in most samples, it was minor pollen (Figure 1). According to the pollen profile, one sample would meet the 15% condition. The accompanying pollen was Myosotis sp. pollen (23.33%), Phacelia sp. pollen (11.03%), and fruit tree pollen (10.26%). Various concomitant pollens (Salix sp. 33% and Brassica sp. 16%) were also confirmed in the study (Jerković et al., 2015). The lowest proportion offspring-flowering trees in Czech honey was represented by hazel pollen, Figure 3. The average amount reached 2.96% and in most cases, s it was minor pollen. Hazel is one of the spring pollen-producing plants. The reason for its low incidence might be climatic conditions or the use of pollen exclusively for the development of honeybee colonies (Odoux et al., 2012). The presence of hazel pollen in honey has also been confirmed in Estonia (Puusepp and Koff, 2014), Germany, Australia (Bibi, Husain, and Naseem, 2008), and Lithuania (Čeksteryte, Kurtinaitiene, and Balžekas, 2013).

The representation of the main summer pollen taxa is summarized in Figure 4. Myosotis sp. pollen had the highest proportion in honey (10.99%), but even so, it had a minor or important minor representation in most samples (Figure 1). Forget-me-not is one of the plants with the highest pollen-producing capacity (Table 1) and for a honey to be classified as monofloral honey, the forget-me-not pollen content must be more than 90% (van der Ham, Kaas, and Kerkvliet, 1999). However, monofloral forgetme-not honey is rare in Europe (Persano Oddo et al., 2004). The occurrence of Myosotis sp pollen in honey has been confirmed by several authors, with a varying frequency and amount of this pollen in honey (Stawiarz and Wróblewska, 2010; Gençay Çelemli et al., 2017; Downey et al., 2005). The second most abundant pollen was pollen of the Umbelliferae family (10.03%), similarly to Myosotis pollen, and in this case, the amount of pollen in honey varies, as reported by individual authors. Some authors consider it minor pollen (Lieux, 1981), some consider it dominant pollen (Marco et al., 2012). In Europe, some species of this family are also found in single-species honey (Daucus Carota, Coriandrum sativum) (Persano Oddo et al., 2004). The percentage of pollen of the Umbelliferae family in the May-June period differed significantly from the July-August period. The differences are due to the blooming period of this family, which is mostly summer and autumn, primarily for taxa important from the beekeeping perspective (Abou-Shaara, 2015).

The *Phacelia sp.* is an agricultural crop with a short growing season well-known to the beekeepers. It can therefore be used as a source of nectar and pollen (**Sprague et al., 2016**) by beekeepers themselves, or it is used in intensive agriculture for green manure (**Titov and Mamonov, 2013**). *Phacelia sp.* pollen was confirmed in honey from the Czech Republic and the average content in honey was (8.68%), but in most cases, it had a minor representation (**Figure 1**). The *Phacelia sp.* pollen occurred mostly in honey from the May-July period, in August honey this pollen was represented less. A statistical difference was demonstrated between May and July. Due to the high pollen-producing capacity, honey with a *Phacelia* pollen content >90 % is considered monofloral *Phacelia* honey (van der Ham, Kaas, and Kerkvliet, 1999). A more recent Polish study also admits a lower proportion of pollen grains (from 68%) in the case of corresponding physico-chemical and sensory parameters (Kuś et al., 2018).

The most well-known summer nectar-producing tree is considered to be the linden (*Tilia sp.*). Linden has a high nectar content but a low pollen content. Therefore, a low proportion of pollen grains (>20%) is permissible for monofloral linden honey (van der Ham, Kaas, and Kerkvliet, 1999). The average content of pollen grains in the Czech Republic was 3.52% and in most honey, it represented minor pollen in honey. This finding is in line with Polish and Bulgarian polyfloral honey (Stawiarz and Wróblewska, 2010; Dobre et al., 2013). In three honey samples, the pollen content was higher than 20%, which may indicate monofloral honey. The secondary pollen in these samples was rapeseed pollen (1.21 – 19.86%), *Phacelia sp.* pollen (15.05 – 21.75%), and *Umbelliferae* pollen (2.69 – 18.88%).

As reported by several authors (Lieux, 1981; Kale Sniderman et al., 2018), Vicia sp. pollen is an important taxon in polyfloral honey, however, according to the study (Stawiarz and Wróblewska, 2010), it is considered a minority representative. In the Czech Republic, its average content of pollen grains was 3.62% and in most cases, it was minor pollen (<3%) (Figure 1). Trifolium sp. pollen was represented in Czech honey on average in the amount of 3.47%. It was minor pollen (Figure 1) in most honey. The percentage found is lower than described in other countries (Jones and Bryant, 2014; Stawiarz and Wróblewska, 2010). Varying content of pollen grains of Trifolium sp., however, was also observed in various localities in Lithuania, wherein some localities the content of Trifolium sp. pollen was even lower (Čeksteryte, Kurtinaitiene, and Balžekas, 2013).

A comparison of the pollen profile of the Czech Republic in 2019 with foreign studies is summarized in **Table 3**. The selection of taxa is limited concerning the availability of information on the pollen profile of honey from abroad. Botanical taxa for which a comparative study is not available are excluded from the table.

CONCLUSION

In most cases, Czech honey has the nature of polyfloral honey. In 2019, the predominantly represented pollen was of Brassica sp. Fruit tree, Umbelliferae, and Myosotis sp. had a higher percentage as well. The amounts of dominant pollen taxa in Czech honey do not differ significantly during the year. Confirmed botanical taxa were present in honey in all monitored months. Differences between months were confirmed only for pollens of Salixaceae, Umbelliferae family, Phacelia sp. and they are in accordance with the blooming time of these botanical species. The occurrence of pollen taxa in the months out of the main blooming season is caused by physiological handling in the hive, which results in the transfer of honey and pollen cells in the honey flow season. The bee handling management of honey and pollen is also affected by climate conditions and the availability of pollen and nectar sources each year. For this reason, the study will be extended to the following years in order to confirm or possibly exclude the conclusions found in the 2019 season.

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