Original Article

BEE BREAD CELLS IN HONEY SUPER DISTORT THE RESULTS OF POLLEN ANALYSIS OF HONEY

Dariusz Teper*
Piotr Semkiw
Piotr Skubida
Mikołaj Borański

Research Institute of Horticulture, Apiculture Division in Puławy, Poland

*corresponding author: dariusz.teper@inhort.pl
Received: 24 April 2018; accepted: 14 October 2018

Abstract

The pollen analysis is currently the only reliable test to determine honey variety, but the results are sometimes burdened with error. The main reason for this is additional pollen that got into honey in a way other than with nectar collected by bees but through the centrifugation of combs containing bee bread cells.

Studies were conducted in 2012 - 2013 on how different numbers of bee bread cells placed in the honey super influence lime honey pollen analysis. Bee bread pollen getting into honey during extraction in centrifugal-force honey extractors was proven to significantly influence the results of pollen analysis. In some extreme cases, it might skew the results so much that correct determination of honey variety by pollen analysis is no longer possible.

Keywords: bee bread, bee bread pollen in honey, pollen analysis

INTRODUCTION

Pollen analysis is currently the only reliable test for determining honey variety and its geographical origin. Maurizio (1951) reported that the Pfister first noticed in 1895 pollen grains in honey that could be used to determine a honey's origin. Zander (1935, 1937, 1941, 1949, 1951) later greatly contributed to the development of melissopalynology. At the beginning, during determine the minimum percentage of unifloral honey, pollen representativity classes reflecting how much pollen of forage plants is present in their nectar they were not taken into account. It was not until Demianowicz (1961, 1962) drew attention that the previous interpretation of the results of melissopalynological analyses was incorrect. She aimed to determine the total number of pollen grains in 1 g (10 g) of pure unifloral honeys obtained under isolators. These studies led to the elaboration of a new pollen analysis method based on pollen indices. Maurizio (1951) and Demianowicz (1961, 1962) brought attention to the possibility of additional pollen getting into honey inside a hive through the centrifugation of combs containing pollen preserved in the form of bee bread.

Bees collect and store pollen in bee bread in separate cells of brood combs and normally no nectar is added to these cells. The pollen is preserved with lactic acid created through fermentation and systematically fed to larvae. Sometimes, bee bread might rarely be covered with nectar when very high nectar flow occurs and a beekeeper fails to add empty brood combs. With insufficient space, the bees fill all the available cells with nectar, including those partly filled with bee bread.

Pollen analysis is currently the only known test for confirming the botanical and geographical origin of honey that is commonly and recommended by the International Honey Commission. This method identifies plant species, including those from foreign climatic zones, visited by bees based on characteristic features of pollen grain structure. The aim of pollen analysis of honey is to estimate the percentage of nectar of individual source plant species based on the percentage of their pollen in honey sediment. For that reason, the pollen grains of anemophil-

ous and entomophilous plants not producing nectar are either omitted in this analysis or counted separately. Including pollen of these species in the calculations would result in lower percentages of pollen of nectar source plants, thus, false analysis results. Due to different pollen contents honeys were divided into five classes (Maurizio, 1939) and in practice into those poor in pollen, with normal pollen representativity and with over-represented pollen.

The limit of pollen percentage was set at 45% for such Polish unifloral honeys as rape, buckwheat and heather honey with normal pollen representativity listed in the withdrawn Polish Standard – Honey (PN-88/A-77626, 1988). This means that if honey contains 45% pollen of these species, nectar content exceeds 50%. The minimum limits for pollen percentages in unifloral honeys differs are regulated by countries and with local documents (Germany - Bekanntmachung von Neufassungen bzw. Änderungen bestimmter Leitsätze des Deutchen Lebensmittelbuches Jahrgang 63, Ausgegeben am Mittwoch, dem 27. Juli 2011, Nummer 111a; Beck & Camps, 2009).

Honeys poor in pollen are obtained from plant species that have flowers built in a way preventing pollen from getting into nectar and/or produce its low amounts. In Poland, such plant species include lime tree (*Tilia* spp.) and locust tree (*Robinia pseudoacacia*). In the lime tree, although nectaries and nectar drops are located at the base of sepals, pollen has limited access to nectar because its inflorescences hang downward and anthers are located far from the secreted nectar on long filaments. The pollen of the locust tree has difficult access to nectar due to the morphology of its flower, as stamen and pistil are tightly enclosed in two accreted petals creating the keel. Anthers crack only after the keel rips, usually during bee visitation of the flower. The results obtained by Maurizio (1949) confirmed that when *Robinia* pollen percentage was high, the total number of pollen grains in 10 g of honey was lower than 20,000 grains in most samples (pollen representativity class I). In addition, pollen efficiency of lime and locust tree flowers is low at 2.7 mg (for *Tilia cordata* - Jabłoński & Kołtowski, 1999) and 1.9 mg (Jabłoński & Kołtowski, 1993), respectively. Due to low pollen amounts in the nectar of these species, pollen percentage limits in unifloral honeys were lowered to 20% for lime honey and to 30% for locust honey (PN-88/A-77626, 1988). Pollen content levels at 20 and 30%, respectively, mean the amount of nectar of these species in honeys exceeds 50%.

Honeys with over-represented pollen are created from plant species with high amounts of pollen in their nectar. This group consists of such honey varieties as chestnut, forget-me-not and eucalyptus which are not obtained in Polish climatic conditions. The chestnut (*Castanea sativa*) is a very valuable bee plant from southern Europe and the high pollen content in its nectar results from numerous anthers that produce the abundance of pollen which easily fall onto the exposed nectar. Another example are the small, tubular flowers of the forget-me-not (*Myosotis*) (Demianowicz & Demianowicz, 1957). Such structure of a flower causes an insect, which trying to access the sweet secretion, pushes almost the entire content of anthers into the nectar. To be called unifloral, the honeys produced plants have to contain at least 90% of the chestnut pollen and 99% of the forget-me-not pollen. The content of the predominant pollen at the levels of 90 and 99%, respectively, means that 50% of honey was made from the nectar of these species.

The results of melissopalynological analyses are sometimes burdened with error due to extra pollen that gets into the honey in a different way than with nectar collected by bee, usually from bee bread.

Only several reports presented the results of both quantitative and qualitative analyses of honeys. The main paper is "The Main European unifloral honeys" (Persano Oddo & Piro, 2004) where, aside from the results of physical and chemical analyses, results of the qualitative and quantitative analyses of 137 samples of lime honey were also presented.

The aim of the research is to assess how bee bread pollen getting into honey influences the results of pollen analysis of unifloral honeys in the case of lime honey.

MATERIAL AND METHODS

Honeybee colonies and sample collection

Studies were conducted in 2012 – 2013 around Puławy, Poland with lime trees as the nectar source. Thirty-one Caucasian (*Apis mellifera caucasica*) honey bee colonies were used in the first year and thirty-two in the second year. The colonies were placed near the small-leaved lime trees (*Tilia cordata* Mill.), the main source of lime yield in Poland, around June 20 when it starts to bloom. Every honeybee colony used in the experiment had a horizontal plastic queen excluder for separate brood chamber and honey super. The colonies were divided into four experimental groups. Combs containing around 200, 800 and 2,000 bee bread cells and none in the control were placed in honey supers. Combs with the bee bread cells were taken from colonies outside of the test not to reduce bee bread stocks in the experimental colonies. After the lime trees stopped blooming around July 10, the produced honey was centrifuged, weighed separately from each colony and sampled for melissopalynological analysis.

Pollen analysis

Every honey sample underwent separate qualitative and quantitative pollen analyses. The qualitative analysis was used to establish the percentage of *Tilia* pollen content in honey. It was conducted according to methodology described in the Regulation of the Minister of Agriculture and Rural Development of Poland of 14 January 2009, which is in accordance with procedures recommended by the International Honey Commission (IHC). The quantitative analysis established the total number of pollen grains in 10 g of honey and was conducted according to methodology recommended by IHC and International Commission for Bee Botany of IUBS (Louveaux, Maurizio, & Vorwohl, 1970).

Statistical analysis

The results were statistically analysed using the Statistica 10 software. For all analyses two-way ANOVA was used. Duncan's multiple range test was applied to determine significant differences between the means. P-value < 0.05 was considered significant. Some data were transformed with Log_{10} or Arc Sin (x).

RESULTS

In the first year, the average total number of pollen grains in 10 g of honey ranged from 3,180 in the control to 12,381 in the experimental group containing 2,000 bee bread cells.

Table 1.

Results of quantitative and qualitative pollen analyses of lime honey samples collected in 2012

Number of bee bread cells in a honey super	n	PG*/10 g of honey from - to	PG*/10 g** of honey the average	% of *Tilia* pollen from - to	% of *Tilia* pollen*** the average	Bee bread pollen
The control	10	2,755 – 4,184	3,180 ^{a}	12.5 – 27.8	20.4 ^{b}	Only single pollen grains
200	7	4,150 – 15,510	7,400 ^{b}	5.9 -16.7	12.0 ^{a}	*Brassica napus, Salix, Prunus* type, *Malus* type
800	7	6,531 – 11,837	8,231 ^{bc}	6.7 – 19.0	12.8 ^{a}	*Brassica napus, Salix, Prunus* type, *Malus* type
2,000	7	9,558 – 19,184	12,381 ^{c}	2.6 – 14.3	8.3 ^{a}	*Brassica napus, Salix, Prunus* type, *Malus* type

* PG – total number of pollen grains (Pollen Grains)

** Log_{10}

*** ArcSin (x)

a, b, c - different letters indicate significant differences at P<0.05

The presence of bee bread in honey supers influenced the results of the qualitative analysis as well. On average, the control contained 20.4% of *Tilia* pollen while percentages of *Tilia* pollen in experimental groups were much lower, dropping to only 8.3% in the group IV (2,000 bee bread cells). According to the Polish Standard, the minimum percentage of *Tilia* pollen required for the unifloral lime honey is 20%. Only the value calculated for the control group (with no bee bread) exceeded this value and was statistically significantly higher than in other groups (Tab. 1). The dispersion graph for the content of *Tilia* pollen in relation to the total number of pollen grains in 10 g of honey clearly shows a negative correlation between additional pollen from bee bread and the percentage of *Tilia* pollen (Fig. 1). Results obtained in 2013 showed even more clearly that the presence of bee bread pollen caused a decrease in the percentage of *Tilia* pollen in honey. The total number of pollen grains (PG)/10 g rapidly grew with an increased number of bee bread cells in the honey super. This influenced on the percentage of *Tilia* pollen in honey to drop from 66% in the control (group I) to only 12% in group IV (2,000 bee bread cells) (Tab. 2).

The trendline visible on the dispersion graph for the percentage of *Tilia* pollen in relation to the total number of PG/10 g of honey additionally proves how much additional pollen from bee bread influences the percentage of *Tilia* pollen in honey resulting in its decrease (Fig. 2).

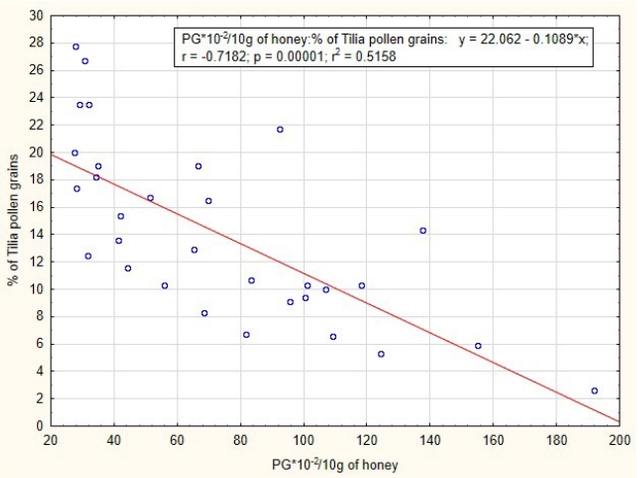

Fig. 1. Influence of increased amount of bee bread pollen on the percentage of *Tilia* pollen in honey in 2012.

Figures 3 and 4 compare the results of qualitative and quantitative analyses for different years, in groups: I – the control (without bee bread), II – 200 bee bread cells, III – 800 bee bread cells, and IV – 2,000 bee bread cells. Clear differences between years are noticeable, but the presence of bee bread pollen still tends to influence the percentage of *Tilia* pollen in honey. Honey yield differed significantly between two years, but no differences were found between experimental groups (Fig. 5). Honey production in 2013 was significantly better than in 2012.

Table 2.

Results of quantitative and qualitative pollen analyses of lime honey samples collected in 2013

Number of bee bread cells in a honey super	n	PG*/10 g of honey from – to	PG*/10 g** of honey the average	% of *Tilia* pollen from – to	% of *Tilia* pollen*** the average	Bee bread pollen
The control	8	1,735 - 6,939	3,833 ^{a}	53.0 – 75.0	66.0 ^{c}	Only single pollen grains
200	8	3,724 - 16,162	9,976 ^{ab}	22.0 – 61.0	42.1 ^{b}	*Prunus* type, *Brassica napus*, *Salix*
800	8	6,633 - 182,448	39,732 ^{b}	7.0 – 41.0	28.4 ^{ab}	*Quercus*, *Prunus* type, *Brassica napus*
2,000	8	16,607 - 267,062	127,415 ^{c}	2.0 – 34.0	12.0 ^{a}	*Prunus* type, *Brassica napus*, *Salix*

* PG – total number of pollen grains (Pollen Grains)

** Log_{10}

*** ArcSin (x)

a, b, c - different letters indicate significant differences at P<0.05

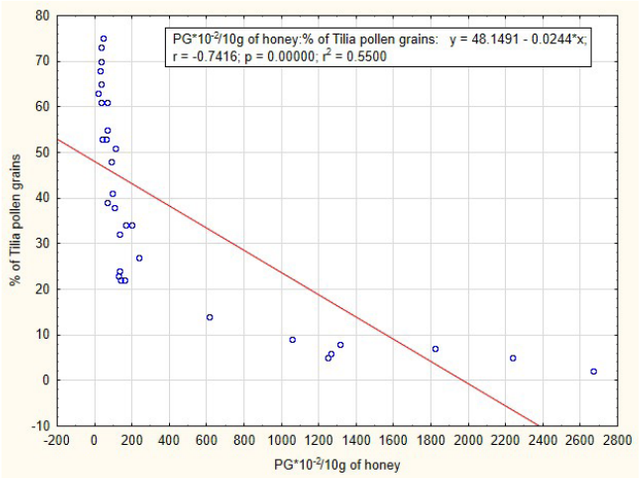

Fig. 2. Influence of increased amount of bee bread pollen on the percentage of *Tilia* pollen in honey in 2013.

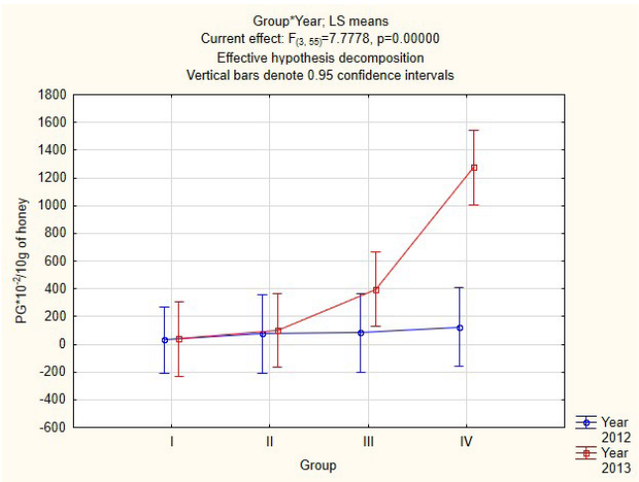

Fig. 3. Comparison of the total number of pollen grains in 10 g of honey in samples from individual experimental years.

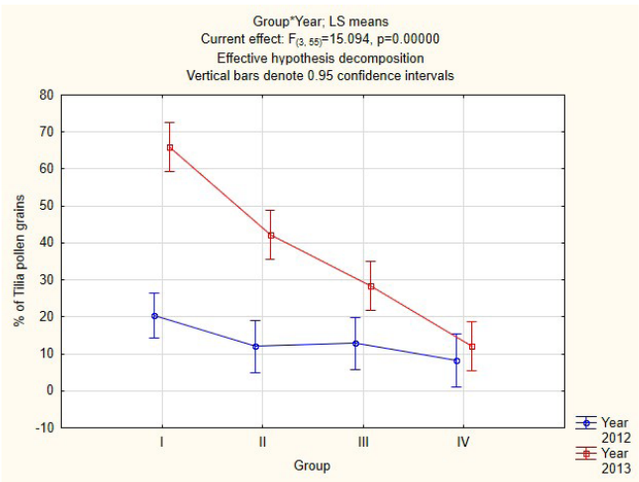

Fig. 4. Comparison of the percentage of *Tilia* pollen in honey samples from individual experimental years.

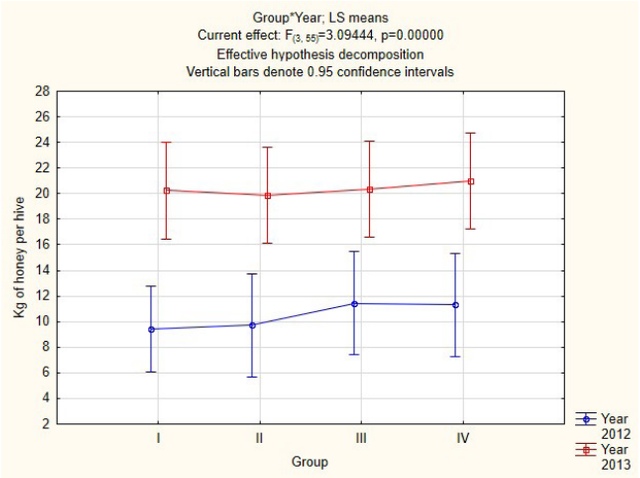

Fig. 5. Comparison of the honey yield between experimental years.

Comparing the results of honey yield and the percent of *Tilia* pollen in the honey from control groups, two years combined, high correlation coefficient obtained (r = 0.71).

Qualitative pollen analyses of the experimental groups containing bee bread cells found significant amounts of *Brassica napus*, *Salix*, *Prunus* type, *Malus* type and *Quercus* type pollen (Tab. 1 and 2). Honey samples obtained from the control group contained no pollen grains from spring plants.

DISCUSSION

Extra pollen most commonly causes the centrifugation of combs containing bee bread cells. Traditionally, beekeepers add frames with a wax foundation during intense bee-colony development in order to prevent swarming. When the colony is strong enough that combs take up the entire body of the hive, in order for the combs foundation to have space some combs are removed from the brood chamber and moved into the honey super. In such cases, beekeepers move combs containing the sealed brood that is usually surrounded with cells containing bee bread. After several days, young bees hatch from these combs, and empty cells as well as those not entirely filled with bee bread are filled up with nectar. Nectar and then honey due to water content causes the bee bread to soften. During honey centrifugation, part or all of the bee bread gets out of the cells resulting in the

addition of pollen to honey. This problem also occurs when queen excluders are not used to separate the brood chamber from the honey super. The results of the quantitative analysis of honeys obtained in 2012 and 2013 were compared which revealed that the total number of pollen grains in 10 g of honey in the first year was significantly lower than in the second year. This was probably caused by unfavourable weather conditions during the lime-tree blooming period which resulted in a lower honey yield and pollen deficiency in bee colonies. With no bee bread reserves in brood chambers, bees probably used bee bread from honey supers, thereby reducing the amount of pollen in honey that was later extracted. Another factor in the smaller harvest of lime honey in 2012 could be alternating flowering of the lime trees that year. Although there have been attempts to develop new methods with state-of-the-art devices, despite its flaws, pollen analysis is still used for determining honey varieties and from which region of the world they originate. The identification of pollen of main nectar source plant species is not that difficult, although it requires experience, but the results are often interpreted incorrectly, because factors influencing them are not considered. Anemophilous and entomophilous plant pollen producing no or very little nectar is often included in calculations of its percentage in honey sediment. However, the biggest mistake is to disregard the extra addition of pollen from bee bread in honey. It is brought to the hive as pollen loads and has no connection to the nectar from which the honey is made. In extreme cases, this pollen can change the classification of honey to another variety if the assessment is solely based on the pollen analysis. Honey should be analysed comprehensively for its physical and chemical, organoleptic and palynological characteristics, and with the results, it is easier to avoid the incorrect determination of a variety.

Proofs of problems connected to extra addition of bee bread pollen in honey can also be found in scientific reports. Persano Oddo & Piro (2004) reported the results of analysing samples of a dozen or so honey varieties which showed a high within-variety discrepancies in both qualitative and quantitative pollen analyses. Honeys declared as lime honeys contained from 1 to 56% of lime pollen and from 3 to 35 thousand pollen grains in 10 g of honey. Organoleptic properties of honeys selected for these analyses were assumed to be typical for lime honeys, but the pollen analyses' results of most samples should have disqualified them. Results obtained in these studies clearly proved that bee bread present in honey supers, using the queen excluders, or not using the excluders, makes impossible to correctly determine honey variety by the pollen analysis. However, it is possible to utilize the results of the quantitative analysis, knowing that some unifloral honeys belong to class I (<20,000 PG/10 g) and the majority – to class II (20,000 – 100,000 PG/10 g), but conducting two analyses significantly increases time and cost of the test. In our opinion, it is necessary to consider if the method of pollen analysis requires any changes that would minimise the risk of false interpretation of its results.

REFERENCES

Beck, G., & Camps, G. (2009). Neue Spezifikationen für Trachthonige. *Deutsche Lebensmittelrundschau* DLR 105 - Februar 2009, 105-110.

Bundesanzeiger - Bekanntmachung von Neufassungen bzw. Änderungen bestimmter Leitsätze des Deutchen Lebensmittelbuches, Jahrgang 63, Ausgegeben am Mittwoch, dem 27 Juli 2011, Nummer 111a.

Demianowicz, Z. (1961). Pollenkoeffizienten als Grundlage der quantitativen Pollenanalyse des honigs. *Pszczelnicze Zeszyty Naukowe*, *5*(2), 95-105.

Demianowicz, Z. (1962). Ocena jednoodmianowych miodów lipowych przy zastosowaniu metody współczynników pyłkowych. *Pszczelnicze Zeszyty Naukowe, 6*(1), 25-45.

Demianowicz, Z., & Demianowicz, A. (1957). Nowe podstawy analizy pyłkowej miodów. *Pszczelnicze*

Zeszyty Naukowe, *1*(2), 69-78.

Jabłoński, B., & Kołtowski, Z. (1993). Investigation of beekeeping value of false acacia (*Robinia pseudacacia* L). *Pszczelnicze Zeszyty Naukowe*, *37*, 65-80.

Jabłoński, B., & Kołtowski, Z. (1999). Nectar secretion of some species and hybrids of lime-tree (*Tilia* L.). *Pszczelnicze Zeszyty Naukowe*, *43*(1), 279-290.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1970). Methods of melissopalynology. *Bee World*, *51*(3), 125-138.

Maurizio, A. (1939). Untersuchungen zur quantitativen Pollenanalyse des Honig. *Mittig. Aus d. Geb. d. Lebensmittelunters und Hygiene*. Bd. XXX, 27.

Maurizio, A. (1949). Pollenanalytische Untersuchungen an Honig und Pollenhöschen. Beih. zur Schweiz. *Bienen-Zeitung* Bd. 2. H. 18. 1949

Maurizio, A. (1951). Pollen analysis of honey. Bee World, *32*, 1-5.

Persano Oddo, L., & Piro, R. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie, 35*(1), 38-81. https://doi.org/10.1051/apido:2004049

Polish Standard – Honey - PN-88/A-77626 (1988) „Miód Pszczeli". Dziennik Norm i Miar nr 8/1988, poz. 19. Wydawnictwo Normalizacyjne Alfa. Warszawa

Regulation of the Minister of Agriculture and Rural Development of 14 January 2009 on the methods of analysis related to the assessment of honey - Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 14 stycznia 2009 r. w sprawie metod analiz związanych z dokonywaniem oceny miodu. Dz. U. nr 17, Poz. 94.

Zander, E. (1935, 1937, 1941, 1949, 1951). Beitrage zur Herkunftsbestimmung bei Honig. Pollengestaltung und Herkunftsbestimmung bei Blütenhonig, Bd. I Berlin, Verlag der Reichsfachgruppe Imker. Bd. II Leipzig, Liedloff, Loth u. Michaelis. Bd. III Leipzig, Liedloff, Loth u. Michaelis. Bd. IV München, Ehrenwirth. Bd. V Leipzig, Liedloff, Loth u. Michaelis.