

Thesis of the Ph.D. dissertation

**THE EFFECT OF THE AGE OF QUEEN BEE ON THE SPRING
DEMOGRAPHIC DEVELOPMENT, THE HONEY PRODUCTION AND THE
VARROA MITE (*VARROA DESTRUCTOR*) BURDEN OF HONEY BEE (*APIS
MELLIFERA* L.) COLONIES**

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1. Background and goals of the thesis

From the point of view of beekeeping production, not only weather factors are decisive, but also production with a bee variety that is well adapted to the ecological conditions of a region is one of the basic conditions for successful beekeeping. In Hungary, beekeeping takes place with the Pannonian bee (*Apis mellifera carnica pannonica*). It has been bred for decades under controlled and defined conditions, and the queen bee is bred on queen bee rearing farms, where the breeding honey bee colonies undergo variety testing every year. However, in addition to the trade in beekeeping products, beekeepers also have to reckon with a possible gene mixing process through international trade in honey bee colonies and honey bee exports and imports. As a consequence, the genetic stock of *Apis mellifera carnica pannonica*, which has developed over the centuries and has been bred through the careful work of bee breeders, may be endangered (Mátray and Molnár, 2000).

Presently, the rate of acceptance of queens has declined, due mainly to the appearance of the Italian honey bee (*Apis mellifera ligustica*) in Hungary. The Italian honey bee worldwide was a popular variety of honey bee (*Apis mellifera* L.), due to their excellent adaptability to different climate conditions and their ability to accumulate large amounts of honey.

In Hungarian beekeeping practice, the queens are changed after two years of age. The performance of a queen changes over time. However, the line of reasoning described above that the acceptance of new queen bees from queen bee rearing farm by the host honey bee colonies is in many cases unfavorable, the question arises as to whether it is really necessary to replace the two-year-old queen bees.

It is important to mention here the fact that in many cases the beekeeping performance of the queen bee is incorrectly judged by the beekeeper, as it is influenced by the combined effect of several factors: successfulness of mating flight, vitality of queen bee and colony, quality of wax.

The anti-mite preparations used in the control of *Varroa destructor*, the ectoparasite of honey bees, which cause the greatest economic damage in Hungarian apiaries, cause the accumulation of residues in wax and honey. As a result, brood can also be “incomplete” on high-chemical wax, but in this case the beekeeper misjudges the performance of the queen bees. In the case of unrelated brood, the beekeeper may look for the fault in the queen bee. As

a result of unfavorable mating of the queen bee, the rate of drone brood increases in the colonies, leading to an increase in the number of *Varroa destructor* mites.

Among the traits examined in the assessment of bee colonies, and thus of queen bee, the tendency to collect is at the forefront, as the direction of production requires it. In second place is fertility, which is closely related to the tendency to collect and the performance of the queen bee.

Thus, the most important property to be examined in the evaluation of the queen is the examination of the ability to lay eggs. The egg-laying ability of a good queen bee also depends on her age and the season, so an excellent queen can be briefly characterized as maintaining a compact, closed, large-area brood appropriate to the season and her age (Plotár, 2003).

The queen bees begin its ovulation activity in February (Örösi, 1957), but then, mainly due to the inhibitory effect of low temperatures, even the most excellent queen lays only a few eggs (KÁTKI et al., 1996).

The initial period of spring demographic development of honey bee colonies is the most suitable for starting the queen ovulation performance study, however, opinions differ significantly in this area as to when it is justified to first open a hive. This time can usually be set in the days following clearing flights (Zimmer, 2018). According to Mátray (2011) it can be done in April, May, while according to Harbo (1988) it is advisable to start the examinations from the middle of March. The first spring inspection of colonies should take place in the second half of March, when the outside temperature reaches 15–18 ° C (Zsidei, 1990). However, in recent years, domestic weather conditions have in many cases not allowed to beekeeper to begin spring monitoring of bee colonies in middle of March. As a result of hive decomposition at low outside temperatures, hatching can catch a cool down, which slows down the development of honey bee colonies.

Experiments show that the proliferation of queen bee is correlated with the yield of honey bee colonies. Decreased performance of the elderly queen (three years or older) causes quantifiable yield loss (Zimmer, 2018). This yield loss can be determined by the amount of honey that can be removed as a function of the age of the queen bee.

During the research, the age of the uterus played a decisive role in all examinations and measurements. It is a common practice among Hungarian beekeepers to require the replacement of two-year-old queen bees. However, due to, the declining acceptance rate, the misjudgment of queen bee performance, the unfavorable weather conditions during mainstream (disregarding ovulation conditions, is the practice adopted so far really justified? In the experiment, such tests and methods were used, which can be easily transferred to the traditional beekeeping practice.

2. Aim of research

During the research the following goals were set:

The main consideration was that spring inspections could be complemented by honey harvest work and anti-mite treatments, with a workflow that could provide more useful information on the condition of bee colonies. In each case, this information was evaluated based on the age of the queen bee.

1. How does the change in the age of the queen bee affect the spring demographic development of honey bee colonies, the date of the population shift?
2. How does intensive spring stimulant feeding affect queen bee performance, does it affect age-effect and timing of population shift?
3. How does the amount of honey harvested change in colonies of different ages of queen bee collected from acacia and sunflower bee pastures?
4. How the number of fallen Varroa mites in the honey bee colonies headed queen bee of different ages changes after the treatments? What factors determine the dynamics of mite fall? It should be mentioned here that the aim of each treatment was not to determine the effectiveness of the anti-mite preparation used.
5. During the economic assessment of replacement of queen bee to determine when the change of queen bee is more favorable at honey bee colony or apiary level. At what age of queen bee and with what acceptance rate of new queen bee can beekeepers except the lowest loss of income?

3. Material and methods

3.1. The location of experiments

The honey bee colonies participating in the experiment were part of an apiary of 200 bee colonies in 2015, where two types of hives could be found (Big–Boczonádi (two–colonies) horizontal lying hives and loading hives with Middle–Boczonádi brood chamber).

During the spring demographic – development the study site was an acacia forest in 2016, then an enclosed garden in the center of Nyírmada (2017–2019).

The location of the apiary was in the Szabolcs–Szatmár–Bereg county, in the administrative border of the Nyírmada settlement (Láczai–forest), where the acacia honey harvest was taken between 2016 and 2019.

The second bee pasture plant on which the amount of honey produced by colonies was examined was sunflower. During the period of collecting from the sunflower, the colonies were transported to Jász–Nagykun–Szolnok county, to a sunflower field administratively belonging to the settlement of Tiszaörs in every experiment year (2015 – 2019).

3.2. The preliminary experiment

In order to start the investigations, it became necessary to map out which type of hive should be used for the investigations. The first comparative study was conducted in July before the start of the PhD study.

Furthermore, the study on the selection of the hive type also sought to answer how the hive type affects the amount of honey that can be taken. An important consideration was whether it is possible to provide a level playing field for bee colonies in the case of hive systems that require different treatment methods. At the time of collected from sunflower, it was possible to study the effect of the hive type on honey production (based on the effect of the age of the queen bee). The study was located in 2015 in Jász–Nagykun–Szolnok county, on the administrative border of a Tiszaörs, approximately took place in the vicinity of a 200-hectare sunflower field. The age of the queen bees was different in the bee colonies involved in the study. The colonies were randomly selected from the apiary. 5 honey bee colonies of Big–Boczonádi (two–colonies) horizontal (lying) hives and 10 Middle–Boczonádi nest hives (loading hives) participated in the preliminary experiment. In the case of loading hives, the birth years of the queen bees were: 2012, 2013, 2014, and 2015, while in the case of loading

hives, it was not possible to include a honey bee colony with a queen bee of 2012 in the experiment. In the case of beehives with loading hives, the birth years of the queen bees were: 2013, 2014 and 2015, respectively. The age of the queen bees could be distinguished on the basis of the color of the queen–marker of the given year. In the honey bee colonies included in the experiment, the queen bees came from controlled queen bee rearing farms, which were established in the region of the beekeeping activity.

The number of experiment honey bee colonies and the age of queen bee examined during the research is shown in *Table 1*.

Table 1.

The number of examined honey bee colonies and the age distribution of the queen bees between 2016 and 2019 in the study

Study year	Age of queen bee	Spring development	Measuring of amount of acacia honey	Measuring of amount of sunflower honey	Treatments against <i>Varroa destructor</i>
2016	0 year	-	-	-	^a 2016 ^b n=20
	1 year	^a 2015 ^b n=16	-	-	^a 2015 ^b n=20
	2 years	^a 2014 ^b n=12	-	-	^a 2014 ^b n=20
	3 years	^a 2013 ^b n=16	-	-	-
2017	0 year	-	^a 2017 ^b n=8	^a 2017 ^b n=8	^a 2017 ^b n=8
	1 year	^a 2016 ^b n=20	^a 2016 ^b n=19	^a 2016 ^b n=19	^a 2016 ^b n=19
	2 years	^a 2015 ^b n=20	^a 2015 ^b n=19	^a 2015 ^b n=19	^a 2015 ^b n=19
	3 years	^a 2014 ^b n=18	^a 2014 ^b n=12	^a 2014 ^b n=12	^a 2014 ^b n=12
2018	0 year	-	-	-	-
	1 year	^a 2017 ^b n=19	^a 2017 ^b n=19	^a 2017 ^b n=19	^a 2017 ^b n=19
	2 years	^a 2016 ^b n=19	^a 2016 ^b n=19	^a 2016 ^b n=19	^a 2016 ^b n=19
	3 years	^a 2015 ^b n=17	^a 2015 ^b n=17	^a 2015 ^b n=17	^a 2015 ^b n=17
2019	0 year	-	-	-	-
	1 year	^a 2018 ^b n=15	^a 2018 ^b n=15	^a 2018 ^b n=15	^a 2018 ^b n=15
	2 years	^a 2017 ^b n=14	^a 2017 ^b n=14	^a 2017 ^b n=14	^a 2017 ^b n=14
	3 years	^a 2016 ^b n=19	^a 2016 ^b n=19	^a 2016 ^b n=19	^a 2016 ^b n=19

Note: ^a Birth year of queen bee; ^b Number of honey bee colonies

3.3. The type of hive used in the experiment from the 2016 study year

Using the experience gained during the preliminary experiment in the further period of the research, it was expedient to use a type of hive. During the research, each of the honey bee colonies included in the experiment was housed in the Middle–Boczonádi loading hive. There are 10 frames in the brood chamber, the size of which was 42x28 cm (including the wood frames). 40x27 cm wax sheet were inserted into the frames. The number of frames in the honey super (honey chamber) was also 10, which are half the size of Big–Boczonádi, which means 42x18 cm frames. Each type of hive used is equipped with a white, hygienic bottom board.

3.4. Spring stimulant feeding of honey bee colonies

The honey bee colonies received different spring stimulant feeding in the 2016 and 2017 study years. The permanent site of the experimental colonies was an acacia forest in 2016, where the pollen required for the spring development of the bees was provided by the orchards and acacia undergrowth in the flying area. The bee colonies were transferred to a closed garden in Nyírség in 2017, which was an area with weaker ecological conditions. Therefore, a stronger spring stimulant feeding was realized. The bee colonies received an antibiotic–free pie once in 2016, while in 2017, two pies and three times a 1:1 sugar syrup contributed to the spring development of the colonies (*Fig. 1.*). In the following years, 2018 and 2019, the last spring stimulant feeding was also realized.



Figure 1. The stimulant feeding (sugar pie and sugar syrup)

3.5. Investigation of the spring development of bee colonies

During the study of the spring development of colonies, data were obtained by recording the number of brood frames. Frames with contiguous, large-scale covered brood area on both sides were calculated in every experiment year (2016–2019). If a frame had a contiguous, covered brood area on one side, it counted as 0.5.

Data were also obtained by determining the ovulation performance of the queen bee in 2018 and 2019 by determining the covered brood area in cm^2 . The investigation period can be attributed to the period described for previous years.

During the determination of the brood area, the so-called a brood-measuring, transparent plastic sheet was used, the grid of which was adapted to the size of the brood chamber frame of the type of hive used. A transparent plastic grid etched in square centimetres is used to visually

calculate the surface area of the brood. Extension of one oblong was 15 cm^2 in the transparent plastic slab (*Fig. 2.*). Size of frames in the Middle–Boczonádi brood chamber was $42 \times 29 \text{ cm}$, the $42 \text{ cm} \times 29 \text{ cm}$ with wood of frame, so actual surface which occupied by bees and when the egg-laying was realized by queen bees was: 40×27 (1080 cm^2 /one side of one frame, which means 3160 cm^2 /frame).



Figure 2. The brood measuring transparent plastic sheet

3.6. Determination of the amount of harvested honey

The concept of the amount of honey that can be removed, extracted, means that in the type of hive with a loading system, the accumulation of honey collected by bees is characteristic both in separate brood chamber and in honey supers (honey chamber). However, the extraction of honey accumulated in the frames of the brood chamber was not typical, in all cases only the honey content of the frames of the honey supers was harvested. By harvesting the honey in

the brood chamber, the brood area can be damaged, which hinders the development of bee colonies. In this sense, the aim was not to determine the total amount of honey produced by the bee colony, but to determine the amount of honey that could be extracted from the honey supers.

The amount of honey produced by colonies was determined twice in each study year: the amount of honey that could be taken was measured during the period of collect from acacia and sunflower bee pastures.

During the measurement, data were obtained by measuring the honey supers one by one, before and after honey harvest. Measurements were made with a digital scale accurate to one decimal place ($d = 0.1$ kg).

In the case of acacia and sunflower honey two harvest usually took place. The dates of honey harvest work between 2017 and 2019 are shown in *Table 2*.

Table 2.

Dates of honey harvest between 2017 and 2019		
Study year	Dates of acacia honey harvest	Dates of sunflower honey harvest
2017	1. 18–19. May	1. 8. July
	2. 25–26. May	2. 15–16. July
2018	1. 3–4. May	1. 10–11. July
	2. 13–14. May	2. 17. July
2019	1. 8. May	1. 13–14. July
	2. 12–13. May	2. 17. July

3.7. Investigation of Varroa destructor burden in honey bee colonies and the anti-mite treatments (2016–2019)

The honey bee colonies were treated for the first time after the last honey harvest (sunflower) (late summer treatment), and in late autumn during the period of reduced brood or broodless status (closing treatment).

Table 3. provides information on the anti-mite preparations used during the study years.

Table 3.

The anti-mite preparation used during the treatments against *Varroa destructor*

Year	Late summer treatments		Closing treatments
2016	In the experimental honey bee colonies	In other honey bee colonies of the apiary	¹ Destruktor 3.2% ² Emulsion application ³ Coumaphos ⁴ Twice treatment at 7-day intervals
	¹ Apivar ² Long-acting preparation ³ Amitraz ⁴ 6 weeks	¹ Taktik ² Smoking ³ Amitraz ⁴ Single treatments	
2017	¹ Apitraz 500 mg ² Long-acting preparation ³ Amitraz ⁴ 6 weeks		¹ Destruktor 3.2% ² Emulsion application ³ Coumaphos ⁴ Twice treatment at 7-day intervals
2018	¹ CheckMite+ ² Long-acting preparation ³ Coumaphos ⁴ 6 weeks		¹ Bee Vital – Hive Clean ² Emulsion application ³ Oxalic acid ⁴ Twice treatment at 7-day intervals
2019	¹ Taktik ² Smoking (with Furetto device) ³ Amitraz ⁴ Four times treatments		¹ Bee Vital – Hive Clean ² Emulsion application ³ Oxalic acid ⁴ Twice treatment at 7-day intervals
	¹ Tik-Tak ² Smoking strips ³ Amitraz ⁴ Double treatments (2 strips/colony)		

¹=name of preparation; ²= construction, type of preparation; ³=active ingredients; ⁴= duration of treatment, repetition

3.8.Economic assessment of queen bee replacement

The main period of queen bee replacement in Hungarian coincides with the flowering of white acacia (*Robinia pseudoacacia* L.). The hungarian beekeeping practical experiences has shown that the acceptance of queen bee in May – June is the most favorable (Zimmer, 2018), however its acceptance rate decreases.

Due to the unfavorable weather in recent years, honey bee colonies have not become productive during the period of intense flowering of acacia. The main bee pasture, the black locust forest is damaged by frost in many regions of country (Bross, 2018). The honey yield available per colony also showed declining trend.

Based on my research the average acacia honey yielded per colony was between 16.3 and 24.4 kg between 2017 and 2019 (depending on the age of queen bee).

According to the factors described below, the question arose as to how the price of queen bee, the percentage of acceptance relates to each other, considering the quantity and price of acacia honey produced. In recent years, both the selling and purchase price of acacia honey have changed significantly (*Table 4.*).

Table 4.

Changes in the purchase and selling prices of acacia honey between 2005 and 2020

Year	Purchase price (HUF/kg)	Selling price (HUF/kg)
2005	430	920–1100
2006	660	1000–1100
2007	600	1050–1100
2008	650	1100–1200
2009	800	1200–1250
2010	1210	1270–1800
2011	850	1400–2000
2012	740	1500–2100
2013	1400	1850–2050
2014	1800	1910–2300
2015	1400–1800	2000–2500
2016	1800	1850–2100
2017	1800–1900	1920–1950
2018	1800	1925–1950
2019	1200	1900–2100
2020	1700	1900–2500

Source: Takács et al., 2015.; I–I.

In the model calculation, it was examined when the replacement of queen bee the most favorable at the honey bee colony level, in a hobby - apiaries (50 colonies), in supplementary beekeeping (approximately 100 colonies), and in professional livestock (honey bee colony of 150 or more). It was calculated with 2020 data for the price of queen bee and acacia honey. The purchase price of acacia honey, considering the current price immediately after the acacia flowering. This is explained by the fact that most beekeepers already cover the costs of transport to sunflower from the sales revenue from acacia honey production. In the case of mated queen bee HUF 3800 was calculated, while in the case of the purchase price of acacia honey per kilogram, HUF 1700 was considered (price of 2020).

3.9. Effects of outer temperature and relative humidity

The aim of current research was to monitor the spring development of honey bee colonies, and great emphasis was also placed on the evolution of the number of fallen mites during mite treatments (based on the age of the queen bee). Both the spring demographic development of colonies and the effectiveness of anti-mite treatments are greatly influenced by outer temperature and relative humidity.

During the period of spring development of colonies and treatments against *Varroa destructor*, changes in temperature and relative humidity were considered in the evaluation of the results. Meteorological data were available from 21 September, 2017. Data from the study years from the 2017 Varroa mite closing treatment period were evaluated in conjunction with meteorological data.

The site of honey bee colonies was located in Nyírmada. Data provided by the nearest meteorological station were analyzed during the research work. The village of Pusztadobos is located in the immediate vicinity of Nyírmada, which has a remote meteorological station. The distance between the apiary and the meteorological measuring station is 2.7 km. Considering the 5 km² flight area of honey bees, the distance of the meteorological station from apiary allowed the provision of accurate data.

3.10. Statistical analysis

Statistical analysis of the data was carried out using a one-way analysis of variance (ANOVA) with Tukey's post hoc test were done using a statistical package IBM SPSS (version 22; 25), US. Variances between groups of queen bee of different ages were compared with Levene-test. (Due to the homogeneity of the variances between groups the Tukey-test was performed to explore the differences between the groups). Measurements were implemented and presented the data as mean \pm standard deviation (SD).

4. Results

4.1. The effect of age of queen bees on spring development of honey bee colonies and population shift

During the 2016 and 2017 study years, in order to monitor the spring development of honey bee colonies, the aim of the research was to map how the spring demographic uptake of colonies develops as a result of spring stimulant feeding. In the two years, the bee colonies received different levels of stimulant feeding, which was justified by the different sites and different ecological conditions.

The results of the spring demographic development in 2016, the number of recorded brood frames can be found in *Table 5*.

Table 5.

Number of brood frames during the colony-development study in spring of 2016 year
Means \pm SD

Birth year of queen bee and number of colonies	18 th March	25 th March	1 st April	8 th April	17 th April	25 th April	1 st May
2013 n= 21	1.6 ^a \pm 0.2	1.9 ^a \pm 0.2	2.4 ^a \pm 0.2	3.6 ^a \pm 0.1	4.5 ^a \pm 0.1	5.5 ^a \pm 0.2	6.9 ^a \pm 0.2
2014 n= 22	1.9 ^a \pm 0.1	2.6 ^b \pm 0.1	3.5 ^b \pm 0.1	4.3 ^b \pm 0.1	5.5 ^b \pm 0.2	6.9 ^b \pm 0.2	7.7 ^b \pm 0.1
2015 n= 16	2.1 ^a \pm 0.2	3.1 ^c \pm 0.2	4.2 ^c \pm 0.2	5.1 ^c \pm 0.3	6.4 ^c \pm 0.2	7.4 ^b \pm 0.2	8.6 ^c \pm 0.2

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test)

The date of the population shift was seen in the 1 May monitoring. At several times, it was observed that in the colonies with the oldest (three-year-old) queen bees, a developmental stage appeared later during the spring population growth.

During the 17 April monitoring, the condition described by Zsidei 1990 was experienced, that is the time of population shift and on 19 May was the first black locust honey harvest, when the last data were recorded.

According to Zimmer (2018), the time of population shift in the honey bee colonies can be dated to the third decade of April, when brood area is extensive but the population is still small. The most striking signs of population shift are that we find old, died bees in front of the hives, despite of a powerful ovulation of queen bees and number of brood frames are high, the population is low in the honey bee colonies.

There was no significant difference between the queens of the year 2015 and 2016 on 29 March and on 10 April. In these bee colonies, on 1 May, there was a large-scale brood area in

6–7 frames, while in the three–years–old queen’s colonies, this condition was detected on 19 May. In the study of 17 April and 19 May each of the data of the three queen-year-groups were different. In 17 April a population shift was experienced in the honey bee colonies. The honey harvest took place on 19 May, when in the colonies with younger queen bees 8–9 frames with brood were experienced.

As in 2016, the colonies participating in the experiment were inspected in the same period in 2017. The data were recorded in 2017 until the period of collecting from the acacia.

The location of the colonies in 2017 was the enclosed garden in Nyírmada. A stronger spring stimulant feeding was performed this year.

(double sugar pie, three times sugar syrup feeding). The development of the number of brood frames is illustrated in *Table 6*.

Table 6.

**Number of brood frames during the colony- development study in spring of 2017 year
Means ± SD**

Birth year of queen bee and number of colonies	16 th March	29 th March	10 th April	17 th April	1 st May	11 th May	19 th May
2014 n= 18	1.9 ^a ±0.7	2.0 ^a ±0.8	2.5 ^a ±0.7	3.6 ^a ±0.5	4.5 ^a ±0.6	5.4 ^a ±0.6	6.5 ^a ±0.6
2015 n= 20	2.7 ^b ±0.5	3.5 ^b ±0.5	4.2 ^b ±0.5	5.4 ^b ±0.8	6.9 ^b ±0.7	7.7 ^b ±0,6	8.4 ^b ±0.7
2016 n= 20	2.7 ^b ±0.6	3.9 ^b ±0.9	4.6 ^b ±0.8	6.2 ^c ±0.7	7.2 ^b ±1.0	8.2 ^b ±0.9	9.0 ^c ±0.8

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test)

During the 17 April inspection, we detected that in the colonies of three–year–old queens the degree of brood significantly fell behind compared to the colonies of the one and two–year–old queens nevertheless, even between the one and two–year–old queen’s colonies one brood frame difference was typical. According to Zsidi (1990) during this period $\frac{2}{3}$ part of frames of brood chamber should be occupied by brood.

On the 19 May, the three groups showed a significant difference during the first black locust honey harvest. The number of frames with brood in the colonies of one–year–old queen bees were 9.0±0.8 (n=20), while in the colonies with three–year–old queen bees, this number was 6.5±0.6 (n=18).

The equable population shift (within our beekeeping), which was detected on 17 April in 2017 (suddenly decreasing population, increasing the number of frames with brood, the death of old

bees at the front of the hives) was precious, which was induced by the spring stimulating feeding of colonies.

However, differing opinions have been published by beekeepers about the effect of spring stimulant feeding (Standifer et al., 1971). Some researchers have found that feeding bee colonies with a sugar solution is useful (Lunder, 1950), while others have not recommended feeding bee colonies in the spring (Miller, 1911; Phillips, 1933). The effects of spring stimulating feeding have been published in the 1930s. The supply of sugar syrup is considered differently by beekeepers (Crane 1950). According to Digges (1936), spring feeding has a stimulating effect on bee colonies and on the work of nurse bees (brood rearing and care), while Hamilton (1945) suggested that, stimulate spring feeding was not required with well-developed honeybee colonies, and poor colonies also did not require it under favorable weather conditions (Free and Spencer- Booth, 1961). Butler (1946) argues that spring feeding has no stimulating effect and can delay spring growth of honeybee colonies. According to Sheesley and Poduska (1968), feeding of bee colonies stimulates the laying of the queen bee, thereby contributing to the development of bee colonies.

It becomes clear that feeding with sugar pie, sugar syrup contributes to the spring growth of honeybee colonies, because even in the colonies with three-year-old queen bees were found an average of 6.5 frames with brood on the 19 May monitoring in 2017.

Published research on the population shift of honeybee colonies is rare. As to the practical aspects of our results, we determined that the number of brood frames at the time of the population shift shows different rates for each queen bee group in both years. This difference was moderated by the stimulating feeding in the middle of May, because in the colonies with three-year-old queens, two-thirds of the frames of the brood chamber were occupied by brood.

As a result of spring incentive feeding, the population shift of honey bee colonies may occur at an earlier time (17 April in 2017; 1 May in 2016), however, the effect of queen bee's age on honey bee colony strength during the period of demographic development is not mitigated. Among the honey bee colonies of different ages of queen bees examined at the same time, the number of frames containing covered brood was significantly higher in the colonies with younger queen bee at the time of the population shift. As a result of increased incentive feeding (application of sugar pies and sugar syrups), the number of frames containing covered brood was significantly higher in the colonies of one-and two-year-old queen bee.

In the years following the 2017 colony–strength study (2018 and 2019), it became necessary to develop a more accurate measurement method, which can be used smoothly in productive apiaries as well, and which can even be integrated into apiary work processes. Current examination was conducted to determine the effects of age of queen bees on the size of brood area (cm²). During the measurements, the number of brood frames was also recorded. The data were recorded four times between 8 April and 22 May in 2018 (*Table 7*).

Table 7.

Number of brood frames during the colony- development study in spring of 2018 year
Mean ± SD

Birth year of queen bee	8 th April	25 th April	7 th May	22 nd May
2015 n= 17	2.8 ^a ±0.5	4.3 ^a ±0.5	4.8 ^a ±0.4	5.4 ^a ±0.5
2016 n=19	3.2 ^b ±0.4	4.7 ^a ±0.4	5.4 ^b ±0.4	5.9 ^b ±0.7
2017 n=14	3.6 ^b ±0.5	4.9 ^b ±0.5	5.8 ^c ±0.6	6.6 ^c ±0.5

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test)

In the first data set (8 April), the number of frames containing covered brood was statistically lower in the three–year–old queen bee–group. There was no significant difference between the one– and two–year–old queen bee–groups.

Based on the data recorded at the 25 April examination, there was no statistical difference between bee colonies with two–or three–year–old queen bees. During this period, the average number of covered brood frames was statistically lower in the colonies headed three–year–old queen bee than in younger (one– and two–year–old) queen bee – groups.

Compared to previous years, the start of the population shift could be detected at a later date (review of 7 May 2018). Significant differences in the period of population shift in previous years in the groups of uterine age of different ages also appeared in 2018.

This discrepancy was reflected not only in the number of frames containing covered brood, but also in its extent (*Table 8*).

Table 8.

The brood areas expressed in cm² in 2018
Mean ± SD (cm²)

Birth year of queen bee	8 th April	25 th April	7 th May	22 nd May	Total
2015 *n=17	605.8 ^a ±137.2	1162.5 ^a ±427.8	1317.7 ^a ±225.7	1540.0 ^a ±314.5	4680.0 ^a ±958.8
2016 *n=19	848.2 ^b ±176.9	1390.3 ^b ±306.7	1790.3 ^b ±386.6	2141.8 ^b ±438.4	6170.5 ^b ±1061.1
2017 *n=14	1108.2 ^c ±264.8	1909.4 ^c ±539.6	2319.3 ^c ±744.8	2744.6 ^b ±931.8	8081.6 ^c ±2091.0

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

*: number of honey bee colonies

Between 8 April and 7 May, there was a significant difference in the extent of the brood area in all three groups. At the last time point, the group with the oldest queen bee had a significantly lower covered brood area compared to the younger (one- and two-year-old) queen bee-groups.

According to the results of the statistical evaluation, the values of the brood area (cm²) were significantly lower with the age of the uterus.

According to the results of the statistical evaluation, the values of the brood area (cm²) were significantly lower with the age of the queen bees.

Similar to the evolution of the number of brood frame, there was a statistically significant difference in the data of 7 May (perception of the initial period of population shift) between the 3 experimental groups.

Temperature and relative humidity data were also considered in the study. In the week from 25 February to 4 March (when the queen bee begins to lay its eggs under favorable conditions), the lowest temperature was -17.1 °C. The following week, from 5 to 11 March, a daily minimum temperature of -16.9 °C was also typical.

In colonies with a three-year-old queen bee, there was hardly any increase in the size of the brood area from 25 April to 7 May. The maximum daily temperature (29.7 °C) was highest in the week of population shift detection (7 May monitoring).

The evolution of weather factors was also variable during the spring development period of 2019 (March – May), but proved to be less extreme than in 2018. Higher-than-usual temperatures were measurable in the first half of March (daily maximum temperature of 20.1 °C between 11 and 17 March), but significant cooling occurred in the second half of the month. The temperature in April was above the long-term average (weekly average temperatures: 12.4 - 15.1 °C). The results of the spring development of 2019, the number of brood frames are shown in the *Table 9*. illustrates.

Table 9.

Number of brood frames during the colony- development study in spring of 2019 year

Mean ± SD

Birth year of queen bee	23 th March	12 th April	28 th April	7 th May	20 th May
2016 *n=15	2.4 ^a ±0.5	3.4 ^a ±0.5	4.1 ^a ±0.6	6.6 ^a ±0.6	8.0 ^a ±0.7
2017 *n=14	3.1 ^b ±0.6	3.7 ^a ±0.5	5.0 ^b ±0.6	7.2 ^b ±0.5	8.1 ^a ±0.7
2018 *n=19	2.9 ^a ±0.6	3.5 ^a ±0.6	5.1 ^b ±1.1	7.3 ^c ±0.6	8.5 ^a ±0.6

Source: IBM SPSS Statistic Program (Version 22.)

a-c: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

**: number of honey bee colonies*

In 2019, a different trend was observed in the number of brood frames than in the previous study years. Based on the results of the 12 April study, there was no significant difference in the number of frames containing covered brood between the three groups of queen bees of different ages. For the 28 April data, this value is statistically lower in the oldest (three-year-old) queen bee's colonies. During the 7 May review, there were already statistically significant differences between all three groups. At this time, there were signs of a population shift.

In order to determine the extent of the brood area, the second method was also performed in 2019, when they were expressed in cm². The results of the measurements are shown in *Table 10*.

Table 10.

The brood areas expressed in cm² in 2019
Mean ± SD (cm²)

Birth year of queen bee	23 rd March	12 th April	28 th April	7 th May	20 th May	Total
2016 *n=19	494.3 ^a ±78.4	651.1 ^a ±153.5	1120.7 ^a ±175.9	1821.3 ^a ±329.1	2359.3 ^a ±356.3	6446.9 ^a ±581.9
2017 *n=14	484.3 ^a ±51.1	893.1 ^a ±91.3	1174.8 ^a ±201.9	2036.5 ^a ±408.7	2424.1 ^a ±399.5	7012.9 ^a ±851.1
2018 *n=15	535.1 ^a ±81.3	947.0 ^b ±75.7	1331.5 ^b ±242.5	1862.1 ^a ±311.1	2489.0 ^a ±334.8	7164.9 ^b ±715.3

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

*: number of honey bee colonies

For the data of 12 April and 28 April, the colonies headed youngest queen bees had a higher extent of covered brood area. During this period, the average temperature increased by 4 °C (from 10.9 °C to 15.1 °C), while the relative humidity increased by almost 20% (from 56.4% to 75.8%). increased to). Based on the results of 2019, with the increase of relative humidity and average temperature values in the examined colonies, the extent of the brood was significantly higher in the colonies with young (one-year-old) queen bees (reaching 1331.5±242.5 by 28 April).

The results obtained during the recording of the number of brood frames and the determination of the brood extension in cm² in 2018 at the time of the population shift proved significant differences for both measurement methods. However, in 2019 there was only a statistically significant difference in the number of frames which containing brood, this difference was not characteristic in the definition in cm².

Based on the results of the spring development study of 2019, although there was a significant difference in the number of brood frames in the younger queen bee's groups at one time, there was no statistically significant difference at two times (12 April and 20 May). In the data of the brood extension expressed in cm², value was significantly higher in the colonies headed youngest (one-year-old) queen bee only at two monitoring date. There was no statistically significant difference between the two- and three-year-old queen bee- groups at any date.

Regarding the practical results, it can be said that as a result of the unfavorable weather during the spring development period, not only the number of brood frames, but also the extension of

the brood decreases significantly as the age of queen bees. Thus, older queen bee may be more sensitive to fluctuations in outer temperature.

In an experiment in Slovenia, where the aim was to select honey bee colonies with suitable characteristics for queen rearing, the brood area, expressed in cm², was examined. Although the study mentioned that the strength of bee colonies is influenced by the vitality and age of the queen bee, their age was not considered in the chosen colonies. So the colonies were not divided into groups according to the age of the queen bee. Based on their results, the average covered brood area was 7061 ± 2813 cm². Based on our own results, it can be seen that similar values were measured in bee colonies with younger queen bees (7012.9 ± 851.1 , 7164.9 ± 715.3). However, in own results, the standard deviations values moved in a smaller interval. This is explained by the fact that while the age of the queen bee was ignored in the study, in the own experiment, the participating colonies were grouped according to the age of the queen bees. Therefore, smaller standard deviations developed. In colonies with queen of the same age, the brood extension is more uniform. Grouping based on the age of the queen be thus provides an opportunity to judge the causes of developmental differences in colonies in the spring during a productive apiary.

4.2.Changes in the amount of honey based on the effect of the age of the queen bee (2017-2019)

It was possible to evaluate the honey production data from the 2017 study year, when the performance of colonies with new queen bees was also measured. The results of the 2017 acacia honey quantity are illustrated in *Table 11*.

Table 11.

Changes in the amount of acacia honey in the studied bee colonies in 2017**Mean±SD**

Birth year of queen bee and number of colonies	First honey harvest (kg/colony)	Second honey harvest (kg/colony)	Total (kg/colony)
2014 n=12	6.9 ^a ± 0.5	9.6 ^a ±0.7	16.6 ^a ±0.9
2015 n=19	13.9 ^b ±0.5	15.3 ^b ±0.6	29.2 ^b ±0.7
2016 n=19	14.0 ^b ±0.6	15.7 ^b ±0.5	29.6 ^b ±0.9
2017 n=8	6.2 ^a ±0.7	12.3 ^b ±1.7	18.5 ^c ±2.3

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

In colonies with a new queen bee, the amount of acacia honey at the time of the first honey harvest was not reached even the amount experienced in three-year-old queen bee's group. The most outstanding honey production performance was measured in colonies with two-year-old queen bees. The average amount per colony was nearly 30 kg (in terms of total honey production data).

The amount of honey harvested was also measured in the four groups of queen bee of different ages during the period of collecting from sunflower bee pasture, the results of which are shown in Table 12.

Table 12.

Changes in the amount of sunflower honey in the studied bee colonies in 2017**Mean±SD**

Birth year of queen bee and number of colonies	First harvest (kg/colony)	Second harvest (kg/colony)	Total (kg/colony)
2014 n=12	7.3 ^a ±0.4	7.8 ^a ±0.6	15.1 ^a ±0.5
2015 n=19	12.5 ^b ±0.6	13.4 ^b ±0.5	25.9 ^b ±0.7
2016 n=19	12.8 ^b ±0.5	12.8 ^b ±0.4	25.6 ^b ±0.7
2017 n=8	14.1 ^b ±0.7	12.3 ^b ±0.6	26.4 ^b ±0.7

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

In the amount of sunflower honey, it was found that the colonies with the youngest (2017) queen bee had the highest amount of honey harvested, while it was statistically lower in the three-year-old queen bee group. During the longer acceptance period of new queen bee, the

intensity of collection decreases in the colonies, which also prevailed in the amount of acacia honey yield. However, outstanding amounts of sunflower honey have been produced by colonies headed new queen bees.

According to Akyol et al. (2007) in colonies with one-year-old queen bee (the youngest queen bee age group in the study) the average honey yield was 26.3 ± 2.89 kg/colony. Based on own results the average amount of honey produced in the colonies with one-year-old queen bee was 26.4 ± 0.7 kg/colony (in the case of sunflower honey yield).

The following year, in 2018, the honey production of one-, two- and three-year-old queen bee's colonies was also examined during the period of collecting from the bee pasture of acacia and sunflower. Data on the amount of acacia honey are presented in *Table 13*.

Table 13.

Changes in the amount of acacia honey in the studied bee colonies in 2018
Mean \pm SD

Birth year of queen bee and number of colonies	First honey harvest (kg/colony)	Second honey harvest (kg/colony)	Total (kg/colony)
2015 n=17	7.8 ^a \pm 1.9	6.5 ^a \pm 1.6	14.4 ^a \pm 2.5
2016 n=19	9.7 ^b \pm 1.2	9.8 ^b \pm 1.5	19.6 ^a \pm 1.9
2017 n=14	12.7 ^c \pm 1.7	12.1 ^c \pm 1.7	24.8 ^b \pm 2.2

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

The amount of acacia honey harvested in 2018 showed a significant difference between the groups. Examination of the relationships between age of queen bee and honey production showed that honey yield in colonies maintained by older queen bees was statistically declining. The effect of the extremely variable weather had an impact not only on the spring development of colonies but also on honey production.

The dynamic spring development of colonies was started during the spring period in 2018 only at the end of March, and considering the 21-day development period of honey bees, due to the prolonged brood period, the development and population of bee colonies were not peak during the acacia flowering period. The loss of the spring population, the loss of a generation, and its later appearance in the colony can also be reflected in the amount of sunflower honey produced (*Table 14*).

Table 14.

Changes in the amount of sunflower honey in the studied bee colonies in 2018
Mean±SD

Birth year of queen bee and number of colonies	First honey harvest (kg/colony)	Second honey harvest (kg/colony)	Total (kg/colony)
2015 n=17	9.5 ^a ±1.5	7.6 ^a ±1.8	17.1 ^a ±2.4
2016 n=19	11.2 ^b ±1.2	10.5 ^b ±1.9	21.7 ^b ±2.5
2017 n=14	13.1 ^c ±1.6	11.2 ^c ±1.4	24.3 ^c ±2.7

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

In terms of honey production, the most striking differences were found in the data measured in 2018. There were significant differences in the amount of both acacia and sunflower honey between the three groups studied. The explanation for this can be found in the fact that in 2018 the effect of weather factors (temperature and relative humidity) was more strongly enforced.

Differences in next year's honey production results are less dominant between groups of queen bees of different ages. Data on the amount of acacia honey in 2019 are illustrated in Table 15.

Table 15.

Changes in the amount of acacia honey in the studied bee colonies in 2019
Mean±SD

Birth year of queen bee and number of colonies	First honey harvest (kg/colony)	Second honey harvest (kg/colony)	Total (kg/colony)
2016 n=19	8.0 ^a ±2.3	9.9 ^a ±2.5	17.8 ^a ±2.6
2017 n=14	9.6 ^a ±2.7	11.2 ^a ±2.8	20.8 ^b ±3.0
2018 n=15	8.3 ^a ±2.1	10.4 ^a ±1.6	18.7 ^b ±2.6

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

In the 2019 acacia honey quantitative data, it was found that there was no significant difference between the amount of honey measured during the first and second harvest. In the total honey production results, the amount of harvested acacia honey was significantly lower in the three-year-old queen bee-group.

Based on the differences observed in previous years, it was found that the amount of honey yield per family decreases with the age of the queen bee. However, in colonies maintained by two-year-old queen bee, the highest amount of acacia honey harvested in 2019 was measured. There was a more statistically significant difference in 2017 in the total honey production results between the queen bee groups of different ages. In contrast, in 2018, this discrepancy occurred at each spinning time. In contrast, in 2018, this discrepancy occurred at each honey harvest date (first and second honey harvest).

In the 2019 data, there was no significant difference between the groups in the first and second honey harvest results, however, there was a statistical difference in the total honey yield. Thus, if the effect of the age of the queen bee is considered, it is always advisable to judge it on the basis of the total honey production results.

In a Slovenian study the research work in also extended to the amount of harvested honey measured in the bee colonies. The average total amount of honey measured was 9.52 ± 6.6 kg / bee colony. The highest value in the studied bee colonies was 27.65 kg / bee colony (Gregorc and Lokar, 2010). In the case of the average honey yield per bee colony, higher values were measured in our study, with lower standard deviation values. The grouping by the age of the queen bee by Gregorc and Lokar (2010) was not considered. In own results, the lower standard deviations were the result of grouping by age of queen. Grouping by the age of the queen bee provides an opportunity for beekeepers to explore the reasons for the differences in yields between colonies.

4.2.1. Conclusions based on the results of spring development and honey production of honey bee colonies

As the age of queen bee progressed the number of covered brood frames, the extent of brood (cm²) and the amount of honey that can be extracted significantly decreased.

The arrear in the population growth of colonies at the beginning of spring development was determined not only the honey production from acacia (which blooming during the spring demographic development of colonies) but also the amount of honey yield on the later flowering bee pasture plants (sunflower).

In the amount of acacia honey that could be taken away, it was found that in those colonies where queen replacement was performed during the period of intensive spring development,

acacia honey production was significantly lower. However, outstanding honey production is expected from later flowering bee pasture from colonies with new queen bee.

4.3. Results of the *Varroa destructor* treatments

The bee colonies in the experiment were not treated against Varroa mite before spring development and the honey production period. Each year after the sunflower flowering was carried out for the first treatment.

In a comparative study in 2016, a single amitraz smoking treatment and long-acting preparation (Apivar) was used during late summer anti-mite treatment.

The efficiency of the basic treatment (single amitraz smoking treatment) in terms of the total number of fallen mites ranged from 12 to 20%. The long-acting preparation had a higher efficiency. About 78-88% of mites were fallen by the six-week treatment.

The experience gained from the results of the 2016 comparative study was used in the following years. Therefore, the late summer treatments were performed with a long-acting preparation. In the last study year, 2019, a two-stage treatment was performed in colonies.

The results of six-week treatment of 2017 are shown in *Table 16*.

Table 16.

**The results of six-week treatments in 2017 (amitraz)
Mean ± SD (Number of fallen mites/colony)**

	1. week	2. week	3. week	4. week	5. week	6. week	Total
*2014. (n=12)	30.25 ^a ±14.7	29.3 ^a ±8.3	23.3 ^a ±7.3	19.2 ^a ±5.4	11.0 ^a ±5.5	2.6 ^a ±1.7	115.5 ^a ±36.2
*2015. (n=19)	30.5 ^a ±12.6	24.1 ^a ±6.2	21.9 ^a ±5.5	17.9 ^a ±6.1	7.5 ^a ±3.9	1.9 ^b ±1.5	103.3 ^a ±23.1
*2016. (n=19)	27.9 ^a ±11.3	19.9 ^b ±9.3	16.3 ^{ab} ±5.9	9.3 ^{ab} ±4.3	3.7 ^{ab} ±2.9	0.9 ^b ±0.9	78.0 ^b ±28.9
*2017. (n=8)	19.8 ^a ±5.5	9.0 ^b ±3.8	12.0 ^b ±5.4	7.8 ^b ±3.1	6.3 ^b ±4.9	0.6 ^b ±0.5	50.6 ^b ±14.5

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

*: birth year of queen bee and number of honey bee colonies

During the statistical evaluation of the data, the number of groups was expanded with a new group (new queen bee – group). The number of fallen mites was also examined in it. After the first week of long – acting preparation, there was no significant difference between the four groups.

However, as the weeks progressed, the number of fallen mites gradually decreased in the younger queen bee – group. In the last week (week 6.), only the group of three-year-old queen bee had statistically significant increase in the number of ectoparasites.

Following the sunflower honey harvest, the colonies continued to receive anti-mite treatments in 2018. A long-acting preparation was used to treatments. CheckMite+, a long-acting preparation with the active substance coumaphos, was inserted into the brood chamber.

There was no significant difference between the weeks in the number of fallen mites. In terms of the total number of mites, it was found that the three groups studied had nearly the same values.

As weeks progressed, the number of fallen ectoparasites showed a decreasing trend in all three groups. The total number of fallen mites exceeded 300 for all three groups. In the colonies with two-year-old queen bee large standard deviation is observed (*Table 17.*).

Table 17.

**The results of six-week treatments in 2018 (coumaphos)
Mean ± SD (Number of fallen mites/colony)**

	1. week	2. week	3. week	4. week	5. week	6. week	Total
*2015. (n=14)	104.9 ^a ±45.9	77.5 ^a ±20.6	69.8 ^a ±16.6	50.4 ^a ±18.7	21.0 ^a ±16.2	11.1 ^a ±6.4	334.7 ^a ±51.4
*2016. (n=19)	103.6 ^a ±30.7	62.6 ^a ±30.0	59.6 ^a ±29.7	64.7 ^a ±24.9	36.8 ^a ±23.3	9.9 ^a ±5.4	337.4 ^a ±104.6
*2017. (n=17)	92.4 ^a ±38.8	81.4 ^a ±32.3	64.2 ^a ±24.4	57.6 ^a ±21.7	35.9 ^a ±21.6	13.8 ^a ±8.8	345.3 ^a ±65.1

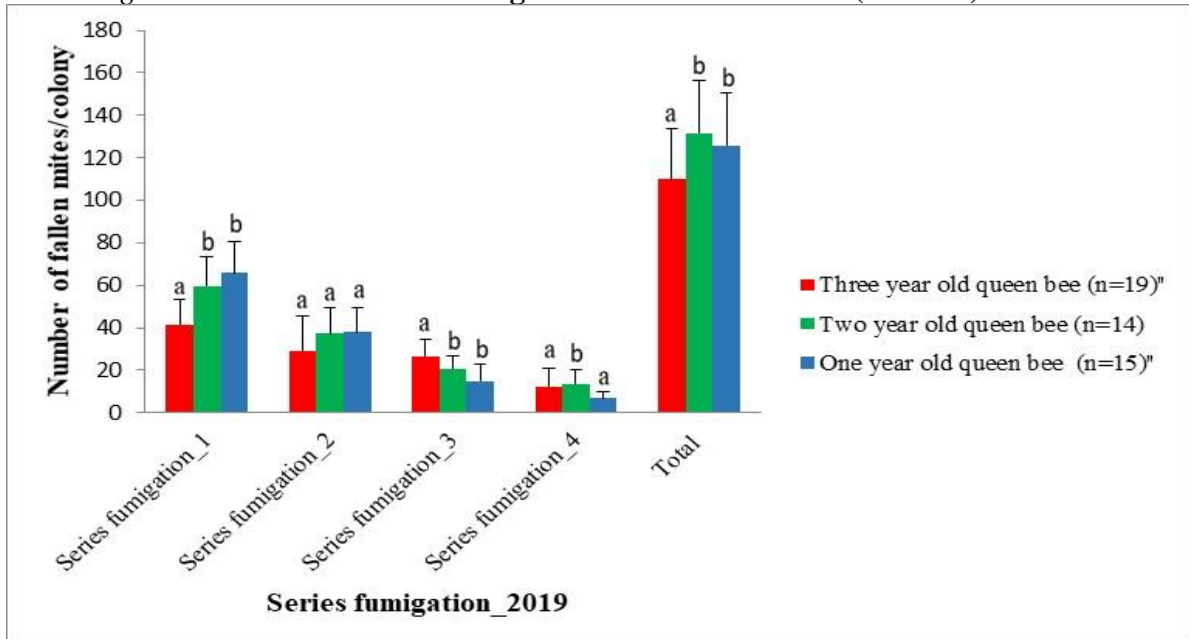
Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

*: birth year of queen bee and number of honey bee colonies

A long-acting preparation was not used for the summer, early autumn treatments of 2019. A two-stage treatment was used during the summer– early autumn period in 2019. During the first treatment, 4 series fumigation and then 2 incense strips were applied with amitraz against the *Varroa destructor* (*Fig. 1. and 2.*).

Figure 1. Results of series fumigation treatment in 2019 (amitraz)



Source: IBM SPSS Statistic Program (Version 22.)

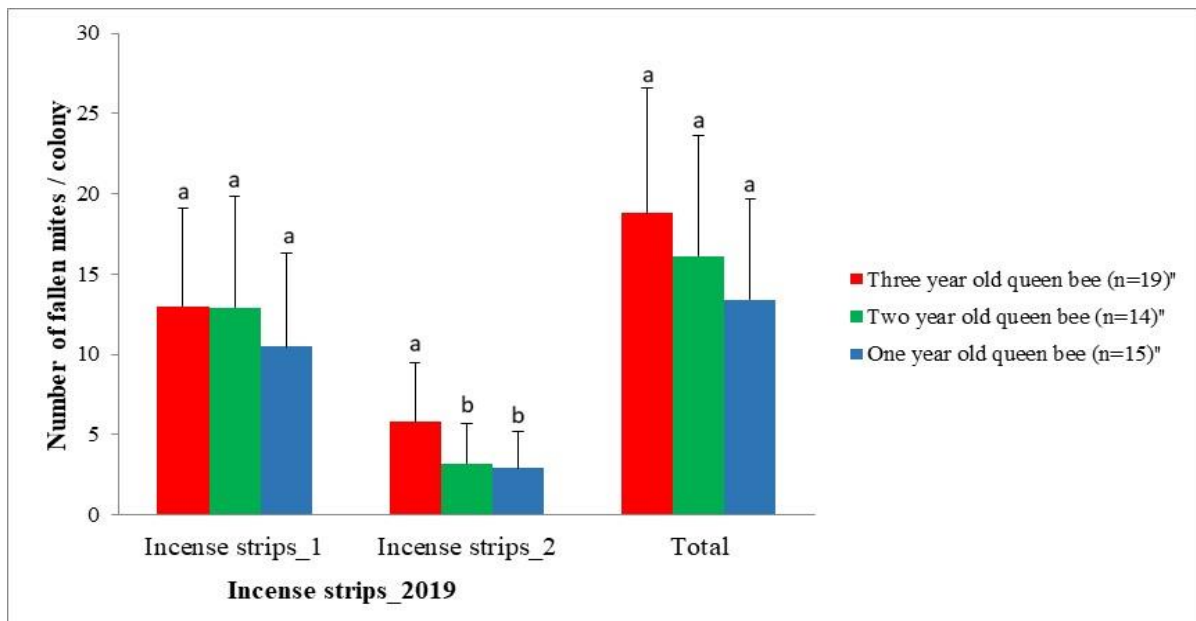
^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

During the first two series smokes, the number of fallen mites was lower in the colonies with three-year-old queen bee than in subsequent smokes. During the third treatment, when the number of mites in the colonies would be expected to decrease, there was a statistically significant increase in the number of mites that fell on the hygienic board. After the fourth smoking, the number of mites in two- and three-year-old queen bee group was still above 10.

In case of the third smoking an increased number of mites was observed in colonies with three-year-old queen bee. In colonies with an elderly queen bee, brood was more loaded with Varroa mites.

Due to the increased number of mites after the third treatments, additional treatments were used. In early autumn period, one incense strip was inserted twice for diagnostic purposes for each honey bee colony.

Figure 2. Results of incense strips treatment in 2019 (amitraz)



Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

After insertion of the first incense strip, there was no significant differences between the groups. However, in the case of the second strip, there were significantly more mites falling on the hygienic board in the colonies with three-year-old queen bee.

In the case of the two incense strips inserted after the series fumigation in 2019, the number of dead mites did not exceed 20. During a 6-week treatment, the number of mites of 20 or less was typical for weeks 5 and 6 in the study years. Thus, it was substantiated that after the series fumigation treatment, further mite reduction was still required during the autumn period (incense strips).

After the application of the second incense strip, the number of fallen mites averaged between 2.9 and 5.8 (with significantly high standard deviations in each group). However, high levels of mite infestation were observed in the colonies during the closing treatments in 2019 (average 87.9–93.1 mites).

The second part of the anti-mite treatments practice was closing (late autumn, winter period) treatments each year. The colonies participating in the experiment were treated with coumaphos in 2016 and 2017 during the final treatment.

In the final treatments in 2016, the colonies were also treated with the coumaphos active ingredients. As the age of the queen bees progressed, the number of fallen mites in colonies increased (*Table 18.*).

Table 18.

The results of closing treatment(coumaphos) in 2016.
Mean ± SD (Number of fallen mites/colony)

Birth year of queen bees and number of colonies	First coumaphos treatment (fallen mites/colony)	Second coumaphos treatment (fallen mites/colony)	Total number of fallen mites
2014; (n=20)	20.1 ^a ±5.2	10.3 ^a ±4.4	31.4 ^a ±8.7
2015; (n=20)	14.2 ^b ±3.7	6.4 ^b ±3.7	20.6 ^b ±6.5
2016; (n=20)	10.1 ^c ±2.6	4.7 ^b ±2.4	14.7 ^c ±3.8

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

Extremely high relative humidity (86.6 and 96.1%) and maximum daily temperature values of 11.1 and 11.3 °C during the 2017 final treatment period with the coumaphos active ingredient (Destruktor 3.2%), the number of fallen mites in the colonies was also high during the second treatment (the average number of fallen mites exceeded 15). This value was significantly ($p < 0.05$) higher in colonies with two- and three-year-old queen bees (*Table 19.*).

Table 19.

The results of closing treatment (coumaphos) in 2017
Mean ± SD (Number of fallen mites/colony)

Birth year of queen bees and number of colonies	First coumaphos treatment (fallen mites/colony)	Second coumaphos treatment (fallen mites/colony)	Total number of fallen mites
2014; (n=12)	17.2 ^a ±8.4	11.5 ^a ±5.2	28.7 ^a ±10.8
2015; (n=19)	14.7 ^a ±6.6	9.3 ^a ±5.1	24.1 ^a ±8.0
2016; (n=19)	7.6 ^{ab} ±4.3	6.8 ^b ±4.3	14.4 ^{ab} ±5.7
2017; (n=8)	7.2 ^b ±5.0	5.0 ^b ±3.0	12.2 ^b ±5.9

Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

According to Bacandritsos et al. (2007) at low outside temperatures and near 100% relative humidity, mite-fallen were slower during treatment with Perizin (predecessor of Destruktor 3.2%) than at higher temperatures. During my treatments, the lowest daily temperature values ranged from -4.1 to -1.4 °C in 2017, compared to -2 °C in the aforementioned study.

The results showed that if the relative humidity is high during the treatment period, the dynamics and rate of mite death in the colonies with the oldest queen bee were shifted (the number of fallen mites is also high during the second treatment).

If the emphasis is on the age of the queen bees, it is supported by the results of the 2017 closing treatment that mites fall is slower in colonies headed older queen bees at low temperatures and high relative humidity. However, this can only be indirectly affected by the age of the queen bee.

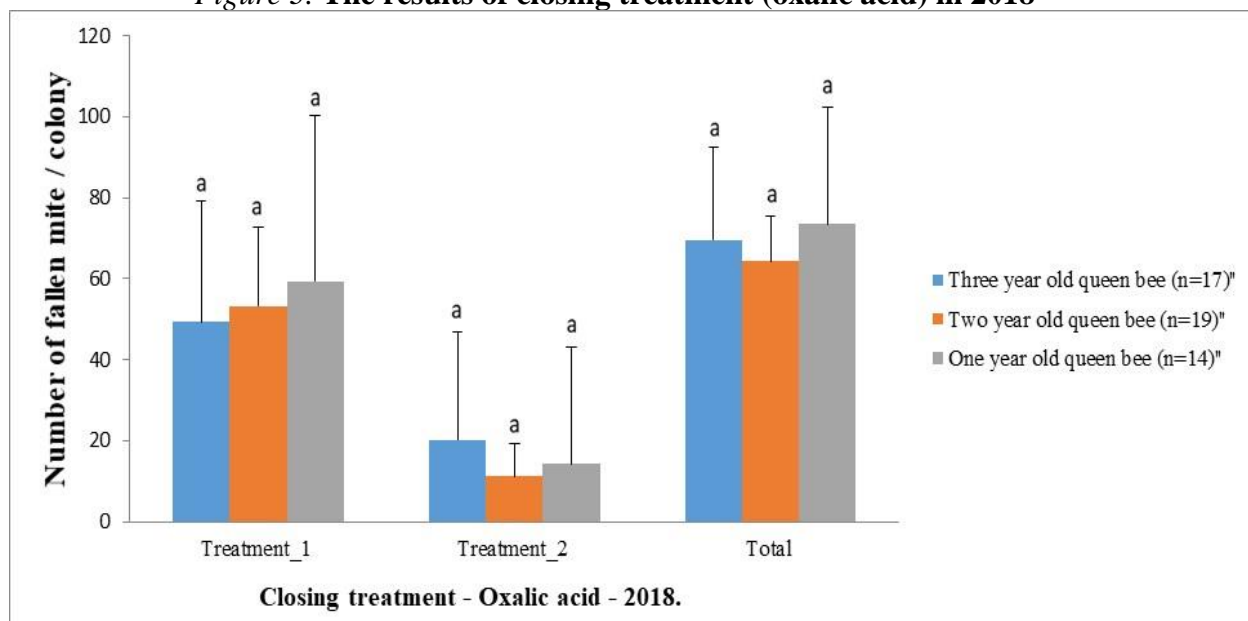
By the time of the closing treatments in late autumn or early winter period, the formation of the wintering cluster in the colonies was already characteristic, where the success of the thermoregulation depends to a large extent on the population and age composition of member of honey bee colonies. These factors were limited in colonies with older queen bees due to declining ovulation performance of the queens.

Bacandritsos et al. (2007) were examined the effects of external minimum, maximum and average temperature and relative humidity during the treatment period with oxalic acid and coumaphos (Perizin) preparation, when the broodless condition was not yet typical. It was found that during oxalic acid treatments the most intense mite-fallen occurred at 60-80% relative humidity. Oxalic acid treatments were performed four times (6 and 21 October and 8 and 26 November respectively). In the days following the third treatment, the average relative humidity was around 80-90%. Intense mite-fallen was only initiated on days 5 and 6 after treatment, when relative humidity decreased to 65%. In the case of the first and second oxalic acid treatments, on the other hand, the number of fallen mites was the highest on the day after the treatments. At the time of the first treatment, the relative humidity was around 60%, and in the case of the second it was below 70%

The results obtained during the oxalic acid treatments showed that the intense mite-fallen was experienced later after the treatments due to the high relative humidity.

Based on the results of our oxalic acid closing treatment in 2018, there were no significant differences in the number of fallen mites in the colonies headed queen bees of different ages (*Fig. 3*).

Figure 3. The results of closing treatment (oxalic acid) in 2018



Source: IBM SPSS Statistic Program (Version 22.)

^{a-c}: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

According to Bacandritsos et al. (2007) the intensity of bee movement was decreased due to low outer temperatures. As a result, the contact of bees with oxalic acid were more moderate. This explained the lower number of fallen mites (in study 15.1 ± 1.63 ; 13.4 ± 0.91).

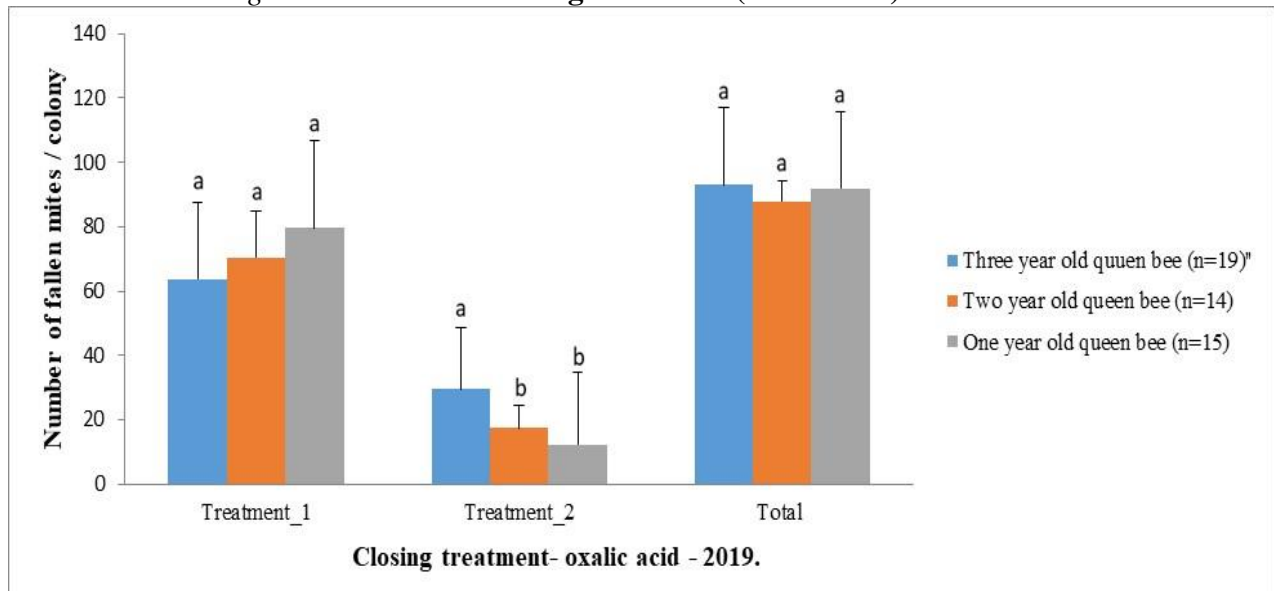
During the period of my treatment (last week of October and first week of November), high daily temperature maximum (22.2 – 23.5 °C) were typical compared to the season. At the time of oxalic acid application, the number of fallen mites during the treatments was significantly higher due to the higher outer temperature (the number of mites exceeded 70 in each group).

During the 2019 closing treatment, the colonies were also treated with BVHC. During the second treatment, it was also found that the number of dead mites was statistically higher in the colonies with the oldest (three-year-old) queen bees (Fig. 4). Mean temperatures ranged from 8.9 to 9.4 °C, while the lowest daily temperature was -5.4 °C during the closing treatment period. The weekly average relative humidity was 91.8%. The results of the previously mentioned study (Bacandritsos et al., 2007) also showed that low temperature and the associated high humidity slow down the mite-fallen during the treatments period.

The results of the closing treatments for the three years (2017, 2018, 2019) showed that the highest number of mites were appeared in 2019 (Table 20). Although in 2017 and 2018 a

preparation with a different active ingredient (2017: amitraz 2018: coumaphos) was used during the late summer, early autumn anti-mite treatment, in both cases it was a long-acting preparation (six-week).

Figure 4. Results of closing treatment (oxalic acid) in 2019



Source: IBM SPSS Statistic Program (Version 22.)

a-c: group means with similar letters are not significantly different at 0.05 level (According to Tukey-test).

A long-acting preparation was not used for the summer, early autumn treatments of 2019. A two-stage treatment was used during the summer-early autumn period in 2019. During the first treatment, 4 series fumigation and then 2 incense strips were applied with amitraz against the *Varroa destructor*.

In the case of the two incense strips inserted after the series fumigation in 2019, the number of dead mites did not exceed 20. During a six-week treatment, the number of mites of 20 or less was typical for weeks 5. and 6. in the study years. Thus, it was substantiated that after the series fumigation treatment, further mite reduction was still required during the autumn period (incense strips).

After the application of the second incense strip, the number of fallen mites averaged between 2.9 and 5.8 (with significantly high standard deviations in each group). However, high levels of mite infestation were observed in the colonies during the closing treatments in 2019 (average 87.9–93.1 mites).

Despite the fact that two-stage mite treatments were applied in 2019, the high number of fallen mites experienced during the closing treatments indicated that it could not replace the effective treatment provided by durable carriers (six-week treatments). Therefore, it is

advisable to choose a long-acting anti-mite formulation after the honey harvest period, to ensure that the number of mites in colonies is kept as low as possible until the time of the late autumn, early winter treatments.

4.3.1. Conclusions based on the results of treatments against varroa mite

Overall, it was found that significant differences were observed not only in spring development, and thus in honey production, but also in the development of mite infestation in older uterine families. This discrepancy was manifested in the following aspect:

1. Mite fallen was different in colonies with queen bees of different ages. In the results of the study years, it was observed that in colonies headed three-year-old queen bees, the death of mites was slower after the treatments.
2. After comparing the results of mite depletion with meteorological data, it was experienced that in colonies with older queen bees, the trend observed in mite-fallen in extreme temperature or humidity conditions were shifted.
3. In colonies with one- and two-year-old queen bees, mite death was more intense during the first treatments, while in many cases, the number of fallen mites increased after the second treatment in three-year-old queen bee-group. It was formulated primarily on the basis of the results of the closing treatment, when the extent of brood was already moderate or broodless period was typical.

4.4. Economic assessment of queen bee replacement

Considering the amount of acacia honey produced between 2017 and 2019, the average honey production results available per honey bee colony and the sales revenue achievable with acacia honey production of one colony are illustrated in *Table 20*. During the average honey production data examined at honey bee colony level, the age of queen bee was also considered.

Table 20.

The average amount of acacia honey produced by one honey bee colony and the available sales revenue based on the age of queen bee

	2017 kg/colony	2018 kg/colony	2019 kg/colony	Average kg/colony	Value of acacia honey produced by one honey bee colony (sales revenue; HUF)
Colony with one-year- old queen bee	29.6±0.9	24.8±2.2	18.7±2.6	24.4±1.9	41 480
Colony with two-year- old queen bee	29.2±0.7	19.6±1.9	20.8±3.0	23.2±1.8	39 440
Colony with three-year- old queen bee	16.6±0.9	14.4±2.5	17.8±2.6	16.3±2.0	27 710

Source: calculated on the basis of own results, 2021.

In the model calculation at the 50% acceptance rate, twice the price of mated queen bee (3800 HUF) was calculated (assuming that the queen bee replaced again by the beekeeper after unsuccessful acceptance). The cost of queen bee replacement per colony decreases with increasing the acceptance rate (*Table 21*).

Table 21.

Examination of the relationship between the acceptance and replacement rate of the queen bee on the basis of the available sales revenue and the cost per honey bee colony

Rate of replacement of queen bee	Acceptance rate of new queen bee (%)					Average acacia honey yield and available sales revenue
	50%	60%	70%	80%	90%	
	Cost per honey bee colony/year (based on replacement of new queen bee) (HUF/colony)					
Annually (100%)	7600 *18.3%	6840 *16.5%	6080 *14.7%	5320 *12.8%	4560 *11%	(100%) 24.4 kg/colony HUF 41 480
Every two years (50%)	3800 *9.6%	3420 *8.7%	3040 *7.7%	2660 *6.7%	2280 *5.8%	(95%) 3.2 kg/colony HUF 39 440
Every three years (33.3%)	2508 *9.1%	2257 *8.1%	2006 *7.2%	1755 *6.3%	1504 *5.4%	(67%) 16.3 kg/colony HUF 27 710

Source: calculated on the basis of own results, 2021.

The data in the table show that there is a difference of 1.2 kg between the average honey production results of colonies with one–and two–year–old queen bee. (Compare to the purchase price of acacia honey in 2020, this means an additional sales revenue of HUF 2040 per honey bee colony. In beekeeping practice at livestock level, a 100% acceptance rate is not or rarely to be expected. No 100% acceptance rate was calculated at honey bee colony level in the study. However, in the calculations for apiaries with several bee colonies, a 100% acceptance rate was considered to determine the exact results.

Considering the average amount of honey produced by colonies headed by one–year–old queen bee as 100%, the average honey production of colonies with three–year–old queen bee shows 33% decreases (67%). In contrast, if the average acacia honey production of two–year–old queen bee colonies comparable to that of one–year–old queen bee colonies, there is only 5% decreases. The annual replacement of queen bee is 18.3% of turnover of a honey bee colony with 50% acceptance rate, while with a 90% acceptance rate it can cause the beekeeper to lose 11% of the turnover.

The sales revenue available with acacia honey is HUF 27 710 in colonies with three-year-old queen bee. These queen bee replacement causes a 9% loss of income. However, the average honey production of one-and two-year-old queen bee colonies significantly exceeds the honey yield available with colonies with three-year-old queen bees.

In the case of queen bee change every two years, which is also accepted in Hungarian beekeeping practice, the following can be experienced: the replacement of queen bee results in a loss of 9.6% in the case of sales revenue with one honey bee colony. (In the case of replacement of queen bee every three-year considering the acceptance rate of 50%: 9.1%). Even with 90% acceptance rate of annual queen bee replacement described above, there is an 11% loss of sales revenue. Changing the queen bee every two years with an acceptance rate of 90% can results in a decrease in sales revenue of only 5.8% at honey bee colony level (change of queen bee every three year: 5.4%).

Table 22. shows the sales revenue of acacia honey production in apiaries with 50, 100 and 150 colonies, considering the average honey yield calculated for colonies with queen bee of different ages.

Table 22.

Development of sales revenue with acacia honey production in apiaries of different number of colonies based on the age of queen bee

	Colony with one-year-old queen bee (HUF)	Colony with two-year-old queen bee (HUF)	Colony with three-year-old queen bee (HUF)
Hobby apiary (approx. 50 colonies)	2 074 000	1 972 000	1 385 500
Supplementary apiary (approx. 100 colonies)	4 148 000	3 944 000	2 771 000
Professional apiary (150 or more colonies)	6 222 000	5 916 000	4 156 500

Source: calculated on the basis of own results, 2021.

The annual replacement of queen bee results in a 20.6% loss of sales revenue in a professional apiary with acceptance rate of 50% (the same loss of sales revenue at honey bee colony level was 18.3%). This mainly causes a significant loss of sales revenue in apiaries, where the majority of colonies have elderly (three-year-old) queen bee, so the queen bee must be replaced.

Based on calculation the available sales revenue of honey bee colony with two-year-old queen bee is almost HUF 4 million in a supplementary apiary and more than HUF 5.9 million at professional beekeeping level. Replacement of biennial queen bee for a beekeeping with 100 or more colonies results in 7.2% financial loss for the beekeepers with acceptance rate of 50%. With 80% acceptance of new queen bee approximately 5.78% loss of income occurs. In a professional apiary the annual replacement of queen bee costs beekeepers approximately HUF 800 000 with an unfavorable acceptance rate (50-60%). In the case of an apiary with few colonies (hobby apiary), where the majority of colonies have elderly queen bee, this cost is 61.7% of the available sales revenue of acacia honey production (HUF 1 385 000 in the model calculation) (Table 23.).

Table 23.

Loss of income due to replacement of queen bee in case of different acceptance rate in apiaries with different number of colonies (HUF)

Acceptance rate of new queen bee (%)	50%			60%			70%			80%			90%			100%		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Annually	285 000	570 000	855 000	266 000	532 000	798 000	247 000	494 000	741 000	228 000	456 000	684 000	209 000	418 000	627 000	190 000	380 000	570 000
Every two years	142 500	285 000	427 500	133 000	266 000	399 000	123 500	247 000	370 500	114 000	228 000	342 000	104 500	209 000	313 500	95 000	190 000	285 000
Every three years	94 050	188 100	282 150	87 780	175 560	263 340	81 510	163 020	244 530	75 240	150 480	225 720	68 970	137 940	206 910	62 700	125 400	188 100

Note: A, Hobby apiary; B, Supplementary apiary; C, Professional apiary
Source: based on own results, 2021.

5. New scientific results

1. As a result of spring stimulant feeding, the population shift in bee colonies may occur two weeks earlier, however, the effect of age of queen bees on colony-strength during the period of demographic development is not mitigated. Among the colonies headed of queen bees different ages examined at the same time, the number of frames containing covered brood was significantly ($p < 0,05$) higher in the colonies with younger queen bees at the time of the population shift. As a result of increased stimulation (application more sugar pies and sugar syrup), the contemporary covered brood covers an average 6.9–7.2 frames in honey bee colonies with one – and two – year old queen bee, while in colonies headed by three–year–old queen bee there is an average of 4.5 ± 0.6 frame with covered brood experienced. After moderate stimulant feeding (single sugar pie insertion) the number of Middle Boczonádi frames involving covered brood in honey bee colonies is on average two frames less.
2. During the spring development period, the March temperature is of the greatest importance. Due to the extremely low temperature (below $-15\text{ }^{\circ}\text{C}$) in March during the spring population growth, in honey bee colonies with three–year–old queen bee the covered brood is concentrated in less than 5 Middle–Boczonádi frames at the time of population shift and the 1320 cm^2 is not achieved by contemporary covered brood. This results in reduced honey production by the later appearance of several generations in colonies (considering the 21–day developmental time of worker honey bees).
3. Three–year–old queen bees are more sensitive to temperature fluctuations during the spring demographic development period than one–and two–year–old queen bees. As a result, the amount of honey harvested decreases as the age of queen bees: a decrease of 10.4 kg in the amount of acacia honey in honey bee colonies. This decreased honey yield does not only occur during collecting from acacia, which blooms during the spring development period. It can also result in 7 kg/colony decrease in the honey yield available on later bee pastures (sunflowers). The extinction of the spring population, or the extinction of a generation, its later appearance in the colony can also be reflected in the amount of honey production.

4. While during spring development the ovulation performance of queen bee is directly influenced by the outer temperature, it only indirectly affects the dynamics of mite-fallen during the treatment against external parasitic mite. The population provided by the queen bee will be a determining factor in the intensity and rate of mite-fallen following treatment. Mite death occurred at slower rate in colonies with three-year-old queen bee, (usually twice treatment; the number of fallen parasites was also significantly ($p < 0,05$) higher during the second treatment); thus, the response of honey bee colonies to anti-mite treatments influences the intensity and dynamics of *Varroa destructor* mite fallen.

6. Applicability of the results

1. In colonies with three-year-old queen bee, the amount of harvested acacia honey is significantly lower. However, as a result of the replacement of queen bee during the flowering period of acacia, the intensity of carrying decreases. So, if the period of acceptance/adoption of the new queen bee overlap period of intensive flowering of acacia, a lower honey yield is expected. In this case, even the amount of acacia honey measurable in three-year-old queen bee-group is significantly ($p < 0,05$) higher than in colonies with new queen bees.
2. Depending on the collecting possibilities of the bee pasture, honey can be harvested twice during the flowering of acacia and sunflower. There are significant differences between colonies in the honey production results obtained during the first and second harvests. The effect of the age of the queen bees on honey production should therefore be assessed on the basis of the total amount honey production results. It is mainly a determining factor during the period of acacia flowering. As the age of the queen bee progresses, honey production decreases significantly. Putting this into beekeeping practice can help beekeepers to choose the right time to replace the old queen bees.
3. Grouping based on the age of the queen bee provides an opportunity to understand the reasons for the differences in the performance of honey bee colonies within an apiary in terms of both spring demographic development and different honey production (amount of harvested honey/colony) outcomes. Furthermore, grouping based on the age of queen bee can also provide useful practicing information in understanding the different mite mortality dynamics between honey bee colonies during treatment period. The age of queen bee in the treated honey bee colony and the climatic factors at the time of treatment against *Varroa destructor* should be considered when monitoring the effectiveness of the treatments.

4. Records made during the demographic development of honey bee colonies in spring and a joint assessment of the age of queen bee provide an opportunity for beekeepers to increase the efficiency of beekeeping production, thereby reducing inconsistencies between honey bee colonies and activities of beekeepers. This evaluation can contribute to the successful implementation of the experience gained by the beekeepers.
5. The annual replacement of queen bee results in 18.3% decrease of sales revenue of acacia honey production at the honey bee colony level. The differences in loss of sales revenue between the two- and three-year-old queen bee changes at 90% acceptance rate is only 0.4% (0.6% at 50% acceptance). Waiting for the third year is not favorable: approximately sales revenue may be 0.5% higher per honey bee colony. The replacement of queen bee every three years could endanger the honey production of following year by increasing the number of honey bee colony losses in the winter period within apiary. This significantly reduces sales revenue: both as a result of replacing lost honey bee colonies and due to a reduction in available honey yields. As a result of increased winter honey bee colony losses fewer producing colonies are available during the honey collection period.
6. In the practice of anti-mite treatment, it is advisable to use long-acting preparation (6 weeks treatment) every year. The long-acting formulations contribute to keeping the number of Varroa mites in colonies low until the time of late autumn treatment. Even with a two-stage summer mite reduction treatment (series fumigation with Furetto device, use of incense strips), the mite removal provided by long-acting miticide product cannot be achieved. The number of fallen mites after serial fumigation and treatments with incense strips is nearly the same for number of fallen mites of 5–6 weeks of long acting preparation. Nevertheless, for the period of closing treatments, Varroa mites were more in colonies treated with serial fumigation and incense strips than after the use of a long-acting product in summer, early autumn.

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Candidate: Marianna Takács
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10057357

List of publications related to the dissertation

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