Limiting the reproductive activity of bee colonies as a biotechnological method for increasing their vitality and productivity

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Abstract. The intensity of development and productivity of the bee colony throughout the entire active period of its life depends on the physiological state of bees and their number in the nest. The tasks set in the work were solved experimentally using the following research methods: zootechnical (selection of groups of analogues, study of the development and productivity of bee colonies), mathematical-statistical (assessment of the reliability of the obtained results). Biometric data processing was performed on a PC using MS Excel software with the use of built-in statistical functions. In the presented work, the data of experimental research on the mechanisms of influence of methods of isolation and replacement of queen bees on the functional state of bee colonies and the dynamics of nectar flow, as well as their functional state after the end of honey collection, was provided. Along with the state of bee colonies and productivity, the degree of development of such an anatomical organ of bees as the fat body was evaluated. During the study, it was established that after the replacement of queen bees with queen cells, the nectar flow decreased in all experimental groups, but after the release of queen bees from the queen cells, the amount of nectar brought by bees increased. A high degree of fat body development was observed among bees where queen bees were isolated. The development of the fat body among the bees of other experimental groups during the experimental period was almost identical. The degree of development of the fat body affected the honey collection of bee colonies. A long isolation of queen bees negatively affects their reproductive capacity. It has been experimentally proven and recommended to use methods of isolation and replacement of queen bees for the period of short honey collections – up to 15 days. Planting a fertile queen bee in a bee colony is the highest-priority option. The proposed biotechnological techniques will make it possible to use the potential of bee colonies, to keep bees with a longer lifespan and to increase the yield of marketable honey per bee colony.

Keywords: bee colony, Ukrainian steppe breed, queen bee, fat body, brood, collecting activity of bees.

1. Introduction

For productive beekeeping it is important to combine all processes of caring for bee colonies with the conditions of the forage resources of the area. Of all the factors that most affect the flight activity of bee colonies and the collection of the forage, a stable honey base is of primary importance (Razanova et al., 2021; Nelson & Jay, 2015, Perry et al., 2015; Brodschneider et al., (2016, 2018); Liolios et al., 2015).

However, the level of productivity of bee colonies is influenced by other factors. Among them, the distance and location of bee colonies relative to massifs of honey plants, ambient temperature, humidity, wind direction and wind power. are important. The flight-collecting activity of bees is closely related to the condition of the nest, namely its size and quality of combs. Factors that affect the development and productivity of bees are multi-vector, they can interact with each other having positive or negative impact on bee colonies. Taking them into account in the technological process of beekeeping helps to optimize the living conditions of bee colonies and ensures their effective use in honey collections (Saranchuk et al., 2021, Uzunov et al., 2017; Requier et al., 2018; Rabie et al., 1983; Radev, 2018).

Nectar-pollinating plants are important in the existence of insects, in particular honey bees. In the conditions of the ever-increasing anthropogenic impact (deterioration of ecology, global warming, reduction of land areas occupied by entomophilous and leguminous crops, etc.) on the environment, there is a threat of a decrease in the number of honey bees (Rumiantsev, 2020; Solomakha & Chornobrov, 2021; Tymochko, 2021).

The goal of the study was the regulation of reproduction of bee colonies through the isolation and replacement of queen bees in the conditions of honey collection from the black locust.

2. Materials and methods of research

The presented research is a part of fundamental study and was conducted in accordance with the plan of scientific research on the topic: "To study the characteristics of trophic links between honey bees and entomophylous plants in the conditions of instability of ecotypes" (number of state registration 0121U108595) throughout 2021.

The study was conducted in the conditions of commercial apiary for honey production in Kyiv Oblast. Throughout the research period the efficient use of honey collection from the black locust (*Robinia pseudoacacia* L.) by bees in the zones of their productive flight was studied. The beginning of the mass blooming was determined by the moment of blooming of 1/4 of all existing flowers. Taking into account the difficulty in enumeration of flowers on the whole tree, for the determination of the moment of the full bloom start, the enumeration of the flowers on the control branches was used. The end of the mass blooming was determined by the status of no more than 25% flowers left on its main branches of first and second order.

For the complex study of the weather conditions of the location of research, the temperature data of the environment from personal observations and Ukrainian meteorological network were used. Describing the average daily temperature parameters throughout the research period their unevenness through the active period should be pointed out, with the sudden changes with visible deviations from optimum ratio of intensive release of the nectar by the majority of plants (22°C).

The blooming of flowers of the black locust started from June 3 at daily average temperature of the environment during the blooming of 19.0°C and lasted 11 days.

Statistical-analytical methods. For a comprehensive assessment of the weather conditions in the research area, the data on the temperature of the external environment from personal observations and the meteorological observation network of Ukraine was used.

The bee colonies met the standard of the Ukrainian steppe breed *Apis mellifera sossimai*, as confirmed by the results of the exterior evaluation. The bee colonies were kept in the long hives for 20 standard frames (measuring 435x300 mm).

Phenological studies, the caring for the bee colonies of control and experimental groups was equal and conducted using a generally accepted method (Brovarskyi et al., 2017). To determine the intensity of development, the state of the bee colonies, and to assess the reproductive capacity of queen bees, the number of capped brood in the nests of bee colonies was counted using a grid frame. The same data show the average daily egg-laying by the queen bee and the sum of the three records of capped brood – the total number of bees in the family. The accounting of the capped brood was conducted throughout the entire period of research. In particular, the strength of the bee colonies was determined by the number of bee spaces occupied by bees, and the number of brood was determined using a grid frame, counting the brood every 12 days.

Bee colonies were selected on the principle of analogues taking into consideration their equality in strength, brood number, stocks of food, origin and age of queen bees (age – 2 years).

The number of open brood raised before capping is calculated using the formula:

$$\mathbf{NoB = degQbx10}$$

where:

NoB – the number of open brood;

degQb – daily egg-laying capacity of queen bees;

10 – duration of honey collection, days.

To perform the task, the four groups of the bee colonies were formed, five colonies in a group: one control and three experimental groups. *The control group* of the bee colonies: the nectar flow from the black locust into the bee colonies was accounted. For the evaluation of the honey collecting conditions of the bee colony, the commodity scales VShP -150 was used. Throughout the whole period of blooming every day in the evening the bee colony was weighted. The quantity of food brought in a day was defined according to the difference in values.

I experimental group (I EG) of bee colonies*:* 10 days prior to the honey collection from the black locust the queen bees were removed and replaced within 30 minutes by mature capped queen cells;

II experimental group (II EG): 10 days prior to the honey collection from the black locust the queen bees were left in the bee colonies, but isolated in the cages of Haydar. The queen bees were released from the cells after the end of honey collection;

III experimental group (III EG): 10 days prior to the honey collection from the black locust the queen bees were replaced by the mated queen bees from the nucleus hives. The accounting of the nectar flow into the bee colony was conducted every five days throughout 25 days, the sampling of bees for identifying the degree of development of the fat body was conducted at the beginning of research, beginning of honey collection, end of research.

For identifying the degree of development of the fat body of bees, from every bee colony, from farthest combs in relation to the center of the nest, 50 bees were selected, euthanized by cold at a temperature 1°C and fixed in 70% ethanol. After the dissection, under the microscope MBS-10, the evaluation of the development of the fat body on a five-point scale was conducted in accordance to the methodology of Mauricio (1958).

The honey productivity of bee colonies was conducted using the gross income of honey (extracted honey and honey left in a hive as a feed stock). The total amount of extracted honey was determined by weighing the bucket filled with the product on the scales VShP -150.

The objectives of the work were achieved using zootechnical (the strength of the bee colony) and statistical (mathematical processing) methods of research.

The data was processed using the generally accepted methods of variational statistics according. Calculations were carried out with the help of computer equipment and application software packages MS Excel – 2000. The probability of difference in indicators was determined by Student’s criteria $P > 0.95$.

3. Results and discussion

Jones (2013, 2015) and Wright (2018) it has been proven that there is a complex relationship between the functional state of the honey bee colony and the biological diversity of entomophilous plants in conditions of instability of ecotypes, which can be traced only in view of the strength of the colony and the intensity of honey collection.

In the productive conduct of beekeeping it is important to connect all the processes of handling the bee colonies with the conditions of the forage resources in the area. To do that, it is necessary to take inventory of entomophylous and polliniferious plants in places of beekeeping, determine the timing of blooming, areas and their productivity. With this information, the beekeeper has the possibility not only to determine the periods that are problematic for beekeeping (the ones without the honey collection) and make adjustments to the system of breeding of bee colonies, but also to develop measures for the effective use of the food sources (Herbert et al., 1977; Loper & Berdel, 1980; Kapheim et al., 2011).

Responding to external and internal stimuli, worker bees create optimal conditions for the development of multi-vector regulation of the processes of forage accumulation and consumption, brood rearing, nest building, etc. The vast majority of researchers studied the reproductive capacity of queen bees under the influence of one or more factors. They did not carry out comprehensive research that would contribute to the expansion of scientific provisions on the substantiation of the combined interaction of external and internal factors on the processes related to the cultivation of brood in the nests of bee colonies, the regulation of their reproduction by isolation and replacement of queen bees. (Potts et al., 2010; Smith et al., 2013; Winfree, 2010; Goulson et al., 2015; Fedoriak et al., 2021).

Having studied the methods of isolation and replacement of queen bees, the optimal terms of their isolation and replacement, the peculiarities of the biological relationship between the physiological state of bees and the collection of carbohydrate feed, it is possible to significantly increase the production yield (Mishchenko, 2021).

Before the beginning of the blooming of the black locust, the bee colonies had the sufficient number of working bees to provide the rearing of the large number of brood. The egg-laying capacity of the queen bees before their isolation or replacement is a fairly high rate for this period (Table 1).

Table 1. The average daily egg-laying capacity of queen bees during the honey collection from the *Robinia pseudoacacia* L., eggs per day.

Rate	**The average daily egg-laying capacity, eggs/day**			
	Control	I EG	II EG	III EG
M ±m	1548.0 ±29.12	1735.0±14.55*	1815.0±28.7*	1854.0±41.2*
Lim	854.0-1996.0	1385.0-1877.0	1080.0-1920.0	940.0-1978.0
Cv,%	19.22	3.68	6.57	7.14
% before the control	-	12.1	17.2	19.8

Note: M±m - average of characteristics and standard deviation, Lim - range of variability, and Cv - coefficient of variability of characteristics within the analyzed groups, *means significant differences in the Student t-test. (P > 0.95).

These data indicate the excess of egg-laying capacity of queen bees in the bee colonies of the experimental groups comparing to the bee colonies of the control group. The differences in the strength of the bee colonies were also observed despite the fact that their groups were formed using the method of analogs. This can be explained by the fact that after the overwintering working bees of these bee colonies had the different degree of body deterioration. For the period of replacing the old bees with the young ones the mortality among physiologically exhausted specimens most likely occurred unevenly, which affected the difference in the strength of the bee colonies for the period of the beginning of research.

In spite of the seemingly minor variance in the egg-laying capacity of the queen bees, the variation coefficient (Cv) for the number of the laid eggs averaged 5.79%.

The black locust belongs to the plants of the first productive honey collection. It produces nectar throughout a day, that is why bees are involved in its collection all day. According to indicators of the control hive, throughout the whole period of blooming of the above-mentioned honey plant bees collected 1600 to 8400 g of the nectar.

Varying age distribution of the bees of the researched colonies influenced the intensity of their flying-collecting activity (Table. 2).

Table 2. The dynamics of strength and productivity of colonies during the honey collection from the *Robinia pseudoacacia* L., (period: 24.05-21.06.2021).

The groups of the bee colonies	**The number of brood before the honey collection, quadrants**			**The number of brood after the honey collection, quadrants**			**Honey productivity, kg**		
	M±m	Lim	Cv	M±m	Lim	Cv	M±m	Control-research	td
Control	126.8±7.47	56-148	40.83	224.4±7.91	180.0-260.0	18.87	17.3±1.07	100.0	-
I EG	128.7±8.63	78-150	24.76	101.4±8.82	47.0-120.0	9.53	23.1±1.54	**33.5**	1.01
II EG	134.6±8.59	66-146	24.48	-	-	-	**31.5±2.48**	**82.0**	1.38
III EG	128.3±0.43	42-145	21.59	**249.8±11.38**	135.0-285.0	21.49	18.4±1.13	6.4	0.8

Note: td – criterion of probable difference; other letter symbols as in Table 1.

Data analysis in I experimental group of the bee colonies allowed to identify the pattern of the nectar delivery depending on the state of the bee colonies. It was identified that after the removal of the queen bees the nectar flow decreased comparing to the control group. But after the exit of the queen bees from queen cells the quantity of the brought nectar increased comparing to the bee colonies of the control group. On the average, bee colonies of the experimental group collected 5.8 kg of honey more comparing to the colonies of control group. In the bee colonies of II experimental group the nectar flow also increased comparing to the bee colonies of the control group. On the average the bee colonies of the II experimental group collected 14.2 kg more that is 82% comparing to the bee colonies of the control group, however there has been the decrease in the strength of the bee colonies of the experimental group due to the long period of the lack of sufficient number of brood. Date analysis of the III experimental group of the bee colonies where queen bees were replaced with the young mated queens also demonstrates the increase in the nectar flow comparing to the bee colonies of the control group. On the average the bee colonies of the III experimental group collected 1.1 kg honey more comparing to the bee colonies of the control group.

According to the results of the conducted research it was identified that the replacement of queen bees with the queen cells in the I experimental group and their replacement with the mated queen bees in the III experimental group had different impact on the brood area. And vice versa, the dynamics of the number of the brood at the end of the honey collection in the II experimental group of the bee colonies was different from the number of available brood at the beginning of the honey collection and decreased by 27.3 quadrants (Table 2). The bee colonies of the III experimental group reached the highest point in the number of brood. These bee colonies after the end of honey collection had the brood that was by 121.5 quadrants bigger than it was at the beginning of the honey collection and were the strongest with the large number of brood of different age.

Another conclusion can be made when comparing the bee colonies of II experimental group. After the end of the honey collection the brood was absent in all bee colonies. However, the isolation of queen bees and lack of brood had no further negative impact on the reproductive capacity of the queen bees.

Along with the state of the bee colonies and productivity attention should be paid to one more indicator, namely the degree of development of the fat body. The results of our comparative evaluation of the degree of development of the fat body of bees of control and experimental groups (Table 3) show that the highest degree of development of the fat body was observed among the bees of the II experimental group.

Table 3. The average values of the degree of development of the fat body of bees of spring-summer generation**.**

The groups of bee colonies	The degree of development of the fat body, points								
	The beginning of research			Before the honey collection			After the honey collection		
	M±m	Lim	Cv	M±m	Lim	Cv	M±m	Lim	Cv
Control	1.2±0.03	1.0-1.6	8.03±1.2	1.8±0.05	1.0-2.2	8.5±1.5	2.2±0.05	1.8-2.9	19.4±2.6
I EG	1.5±0.04	1.0-2.1	10.1±7.8	2.0±0.05	2.0-2.2	12.4±8.3	2.6±0.05	2.0-3.0	17.4±2.2
II EG	1.2±0.04	0.8-1.4	8.04±1.2	2.1±0.06	2.0-2.3	14.0±1.9	**3.2±0.04**	2.0-3.5	9.5±1.2
III EG	1.2±0.04	1.0-1.4	8.03±1.2	2.1±0.07	2.0-2.5	14.2±1.4	2.1±0.06	2.0-2.4	18.9±3.5
td	0.999								

Note: symbols as in Table 2.

As shown in Figure 1, the adipose tissue is single-layer, with the small number of folds, the cells are white, rounded, without visible inclusions. This meets the criteria of the third degree of development. From the moment of isolation and up to its ending in the colonies of this group the physiologically young bees were formed that were able to collect nectar for a long time.

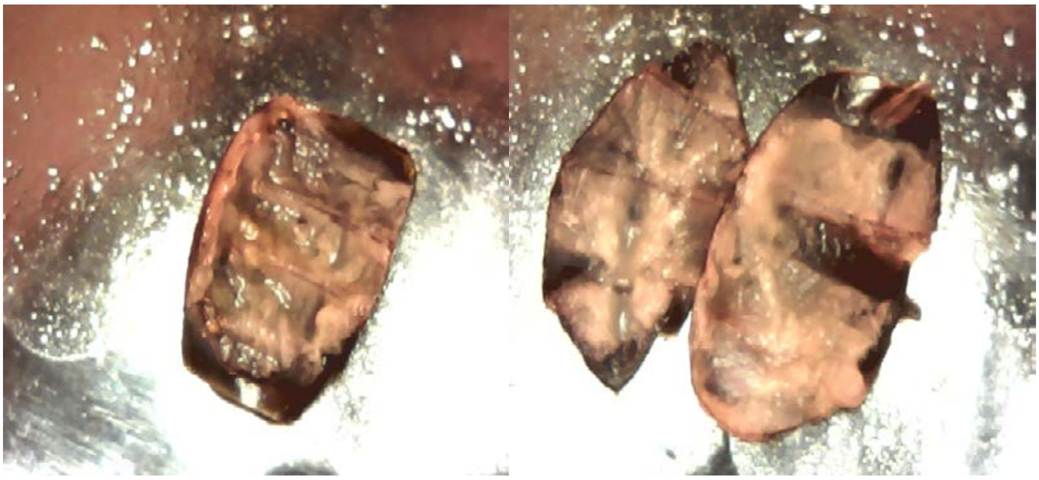

Figure 1. The dissected terga of the abdomen of bees of II experimental group of summer generation

The development of the fat body among the bees of other experimental groups throughout the research period lasted almost identically but was lower on the average by 0.6-1.1 points (Fig. 2).

Figure 2 presents the dissected terga of the abdomen of bees of I and III experimental groups with poorly developed fat body. The fat body of bees is underdeveloped, the chitin of the tergum is clearly visible through it, which according to Mauricio (1958) corresponds to the second stage of development.

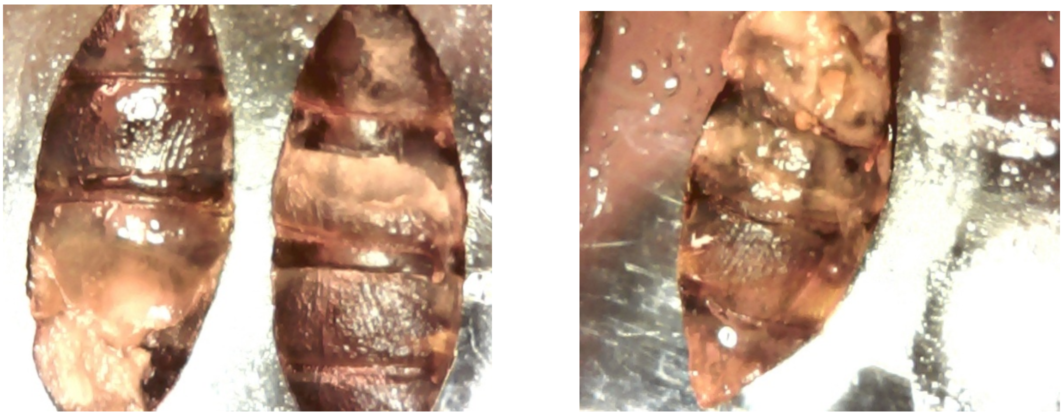

Figure 2. Dissected terga of the abdomen of bees of the I and III experimental groups of summer generation

4. Conclusions

The method of replacement of the queen bee by installing the developed queen cell should be implemented in the conditions of short honey collection in the area of the apiary location. The limitation arises from the fact that after the birth of the young queen bee and up to the beginning of her laying the first eggs the location of the hive should not be changed. Moreover, the bee families with working mated queen bees don’t always accept the queen cell, that is why the result of implementing such method of the replacement is difficult to predict accurately. Therefore, the replacement of the queen bees using this method should be conducted only with the flow of nectar up to 1-0.5 kg into the nest. It is advisable not to use this method in case of weak honey collection since the installing of the queen cell into the bee colony can provoke entering into the swarming state.

Installing the mated queen into the bee colony is the most prioritized option since in this case the strength of the bee colony remains almost unchanged. The existence or non-existence of the favorable conditions for the guaranteed acceptance of the queen bee by the bee colony is a determining factor for the selection of the terms of installing of the queen bee. The replacement of the queen bee by the mature sealed brood is not optional.

A certain pattern in the dependency of the nectar flow on the state of the bee families during the honey collection from the black locust was identified. After the replacement of the queen bees with the queen cells the nectar flow decreased in all research groups, but after the exit of the queen bees form the queen cells the quantity of the brought nectar increased.

The bee colonies of the II experimental group collected 14.2 kg honey more, namely, by 82% compared to the bee colonies of the control group. This indicates the advantage of isolation of queen bees for the period of preparation and use of honey collection lasting up to 20 days.

The high degree of development of the fat body was observed among the bees with isolated queen bees and accounted for 3.2 points. From the moment of isolation of queen bees and up to its end, in the colonies of these groups the physiologically young bees were formed, that were able to collect the nectar for a long period of time. The degree of the development of the fat body also influenced the honey collection of the bee colonies.

In the bee colony, which includes tens of thousands of bees, the function of reproduction is performed by one developed female – the queen bee. Queen bee pheromones constantly affect worker bees, inhibiting the formation of eggs and distracting them from laying queen cells.

Regulation of reproduction is peculiar to all organisms and is associated with the need to prolong life and preserve the gene pool. Temporary cessation of reproduction or temporary isolation of the queen bee is a natural phenomenon that the bee colony needs in order to survive.

As a rule, the honey in the nest is located in the upper part of the combs, and the brood is located below honey. In the presence of a significant amount of nectar brought to the nest, the honey part of the comb increases from top to bottom, reducing the place for laying eggs by queen bee. Thus, bees independently, for some time, temporarily limit the reproductive capacity of queen bee. This provided an impulse for us to study this biological phenomenon.

Effective management of bee reproduction can be achieved when the queen bee is unable to lay eggs, but still has contact with bees. In the isolator the bees take care of the queen bee and receive pheromones. Conditions have been created for the queen bee to stay in the colony with all the signs of its presence, except for the possibility of laying eggs. A colony without brood, but with queen bee in excluder, works no worse than those in which the queen bee moves freely. It was confirmed by the results of our research. The use of the queen excluder provides the opportunity to make significant changes to the technology of keeping bees in order to increase their productivity.

With a strong and short-term honey collection, as from black locust (*Robinia pseudoacacia*), a large number of larvae in the nest reduces productivity indicators. In order to prevent this from happening, we resorted to a temporary artificial limitation of the egg-laying by queen bee for a period of no more than 15 days. Under such conditions, bees maintained high efficiency and their main work was focused on collecting nectar and processing it into honey. In our case, such a short term of isolation as 15 days did not subsequently affect the reproductive capacity of queen bees.

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