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EFFICIENCY OF BEE QUEEN REARING DEPENDING ON ORGANIZATION WAY OF THE NEST IN CELL STARTER COLONIES

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Abstract

The purpose of this research was to reveal the effective methods of directed (artificial) queens rearing depending on organization way of the nest in cell starter colonies. The study object were the honeybee colonies from the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova. To reveal the efficient methods of organizing the cell starter colonies nest for the queens rearing, a special experiment was carried out with 4 similar batches of bee families, with 5 families in each batch, which were different by way of nest structure. The bee families of the I batch served as a control and their nest was organized according to the standard method: in the queenless nest, populated in abundance by bees, 2 food combs (honey+bee bread) and the queen-cells bar frame with 55-65 grafted larvae at the age of 1 day, depending on the rearing series. In addition to the food combs and the queen-cells bar frame, 2 brood combs of all ages in the starter colonies of the the batch II were introduced, 2 sealed brood combs were introduces in the starter colonies of batch III and in the starter families of batch IV - 2 unsealed brood combs were added. The analysis of the research results allowed us to conclude that the presence of the brood in the combs introduced into the cell starter families has a beneficial effect on the final efficiency on the directed queens rearing. The introduction into the cell starter colonies of brood combs of all ages contributes to the obvious increase of the larval acceptance level of 2.5 times (t_d=25.5; P<0.001) and of the yield of obtained queens by 2.9 times (t_d=28.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced. The introduction into the cell starter colonies of unsealed brood combs contributes to the obvious increase of the larval acceptance level of 2.3 times (t_d=20.7; P<0.001) and of the yield of obtained queens by 2.7 times (t_d=30.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced. The introduction into the cell starter colonies only of the sealed brood combs contributes to no significant growth of the larval acceptance level by 4.3% (t_d=1.8; P<0.1) and of the yield of obtained queens by 5,3% (t_d=3.0; P<0.01), compared to the colonies in the control batch, where no brood combs were introduced.

Key words*: rearing, queens, cell starter colonies, combsi, brood, larval acceptance, yield.*

INTRODUCTION

Over the centuries, beekeepers, both amateur and professional ones, have been constantly concerned with the problem of directed queen rearing, both for setting up new families and for replacing of old queens. In 1565 Jacob Nickel was the first in Europe to describe how honey bees can raise queens from worker eggs or very young larvae (Fert, 2011; Büchler et al., 2013). Based on this postulate, beekeepers have further developed a number of methods and proceedings for the directed rearing of queen bees. Already in 1853, the great beekeeper Lorraine Langstroth (quoted by Fert G., 2011) has developed the method of replacing the queen through the process of transferring a natural queen cell to an queenless swarm. In 1883 beekeeper Henry Alley et al. (quoted by Fert G., 2011) has invented the technique for initiating the directed queens rearing using the "closed starter" method, which is used even today. The development of modern queen rearing techniques started in the 19th Century. Gilbert Doolittle (1889) in the USA developed the technique of grafting one-day larvae to start the rearing of future queens. This technique is used until present under the name of the method that bears his name (Fert, 2011; Büchler et al., 2013).

In the scientific literature of the last decades a wide range of biological and technical issues have been approached, regarding the directed (artificial) rearing of *Apis mellifera* queens (Nuru et al., 1999; Kruk et al., 2002; Genc et al., 2005; Kaftanoglu et al., 2011; Büchler et al., 2013; Wubie, 2014; Alber, 2015; Pătruică et al., 2016; Parvu et al., 2017).

A remarkable contribution to the science of artificial rearing of bee queens was brought by renowned german researchers Ruttner H. (1980) and Ruttner F. (1980), who developed the theory and practice in this field. Along with these researchers may be noted and their contemporary Weiss K. (1980), who described in detail the influence of growth factors on the development of queens.

Among the many methods known in the field, as a standard method for enterprises specialized in the queens rearing, it has become the method of grafting one day larvae and their growth from the beginning, during 24 hours in queenless cell starter colony, with subsequent passage of queen-cells bar frame in complete and quite powerful cell raising colony ("cell finishers") (Siceanu, 2012). The success of larvae acceptance, the efficiency of using cell starter and raising colonies ("cell finishers") depends on a number of important technological factors, including the way of brood comb organization is very important. In the standard technology of organizing the cell starter colony, honeycombs with bee bread and honey, before to be introduced into the empty hive body, are pre-stored in the apiary storehouse, so that any potentially competing larvae or eggs may die, so there is no possibility that bees will grow random queen cells. All the bees, left after removing the swarm with the queen, are shaken over this beehive structure, being left instead of the initial family. In this case, the amount of bee in the cell starter family should be as high as possible, at least 0.5 kg on comb. Ensuring such an abundant amount of bee in the starter family, where the brood is totally absent, is a matter not so much technological as biological.

Famous researcher Hans Ruttner (1980) has mentioned: „It would seem that all the royal jelly is given only to the larvae of the queen-cells,and the larvae of workers (which dosn't exist) don't receive it, but it is not so. Production of royal jelly to a colony with unsealed brood decreases. Thus, the first influx of royal jelly to wild queen-cells is lost". For these reasons, the author recommends Bessonet's proceeding, which consists of the following: a Langstroth hive body whose bottom is made of jute mesh is provided with enough bees, from a very strong colony, and with honeycombs and pollen. In this space two unsealed brood combs, are introduced "because it is more natural" (after Bessonet, quoted by Ruttner H., 1980). This corresponds to Laidlaw's recommendation (quoted by Ruttner H, 1980), that on one side of the growing frame there is a unsealed brood comb and on the other side a pollen comb. After 5 hours of formation of this unit, a frame with 28 wet grafted queen-cells is placed in two free beeways between combs. After 24 hours the queen-cells bar frame with the accepted larvae is removed and introduced into the cell raising colonies ("cell finishers"). Each cell starter colony must have at their disposal two cell raising colonies.

In the same paper, the author (H. Ruttner, 1980) describes another proceeding for the growth into the cell starter colonies of grafted larvae, using the Laidlaw-Eckert method, wich involves the use of 1 box (body) and 5 frames, that has a large ventilation space, but without an entrance. In this body are put 3 unsealed honey combs, pollen and some water. Two beeways between combs remain free for queen-cells bar growth frames. In this brood box starter, 2-3 kg of nurce bees are brushed from the brood comb. First, the box, without queen cells and with liquid food, is kept for 3-5 hours in coolness and in the dark. Some beekeepers are putting an unsealed brood combs in this two beeways during repose, which then are removed out.

The larvae are grafted in 60-90 artificial queen cells, the queen-cells bar frames are introduced and left for 24 hours, after that are transfered in the "cell finishers" colonies.

Thus, from the bibliographic analysis of the organization methods of the cell starter colonies, for the growth of the artificial queen cells with grafted larvae, we have found that in some cases in the starter nest, in addition to the queen-cells bar frame, combs with unsealed brood are introduced, whereas in the standard technology of the starter family nest formation, introducing of the brood combs is not provided, according to the Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011.

We can suppose that both, the above-mentioned methods and the standard organization

technology of the starter families includes both, positive aspects (advantages) and negative aspects (disadvantages).
In this context, the purpose of this research was to reveal the effective methods of directed (artificial) queens rearing depending on organisation way of the nest in cell starter colonies.

MATERIALS AND METHODS

The study object were the honeybee families from the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova.

To reveal the efficient methods of organizing the cell starter colonies nest for the queens rearing, a special experiment was carried out with 4 similar batches of bee families, with 5 families in each batch, which were different by way of nest structure, according to the following scheme (Table 1).
The bee families of the first batch served as a control and their nest was organized according to the method elaborated by us (Cebotari V. et al., 2010) for the Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by GD no. 306 of 28.04.2011.

Table 1. Scheme of the nest organization of cell starter colonies for the rearing of queens

Batch number	Nr. of colonies (N)	The order of organization of the combs introduced into the cell starter colonies
Batch I (control)	5	1 food comb (honey+bee bread), queen-cells bar frame, 1 food comb
Batch II	5	1 food comb, 1 brood comb of all ages, queen-cells bar frame, 1 brood comb of all ages, 1 food comb
Batch III	5	1 food comb, 1 sealed brood comb, queen-cells bar frame, 1 sealed brood comb, 1 food comb
Batch IV	5	1 food comb, 1 unsealed brood comb, queen-cells bar frame, 1 unsealed brood comb, 1 food comb

For the organization of the cell starter colonies, the healthy, strong families and with a lot of young bees has been chosen. From these, the queens (queenless) with 1-2 brood combs and bees were removed to make another swarm. The bees of the rest of the combs, from each separate family, were shaken in separated bodies of the hives.
At least after3 hours, 2 food combs (honey+bee bread) and 1 queen-cells bar frame, with grafted larvae were introduced in these hives. The amount of bee per beeway frame (including the queencells bar frame) for each family was at least double and was for Dadant frame not less than 500 g. Thus, in the starter families of the batch I - control were no combs with brood.
Unlike the control group, in the cell starter colonies of the II, III and IV batches, 2 brood combs, different by age, were introduced. Thus, in the starter families of batch II were introduced 2 brood combs of all ages, in the batch III there were introduced 2 sealed brood combs and in the starter families of batch IV 2 unsealed brood combs were introduced. 3 series of grafted larvae per cell starter family were initiated for growing.
The larvae growth time in queen-cells bar in the starter family was 24 hours and the number of accepted larvae and the acceptance rate (%) were then appreciated.
After that the queen-cells bar were transfered in the "cell finishers" colony formed in similar batches, according to the above-mentioned (GD RM no. 306 of 28.04.2011).
In each starter family in all experimental groups, in each rearing series the same number of one-day larvae was grafted.
In the first series of rearing (on 24.04.2017) a queen-cells bar frame with 60 grafted larvae was introduced, in the second series (on 04.05.2017) on queen-cells bar frame were grafted 65 larvae, in the third series (14.05.2017) - 55 larvae were grafted on the queen-cells bar frame.
After the queens emerged in the cell raising colonies ("cell finishers"), the final result was appreciated - the number and percentage (yield) of queens obtained from the grafted larvae in each cell starter family and average on the starter families in each experimental batch.
The obtained in experience data were statistically processed using computer software "STATISTICA - 12" and evaluated their

certainty, according to variation biometric statistics, by methods of Plohinskiy (1989).

RESULTS AND DISCUSSIONS

The research results have shown that the efficiency of queen rearing is determined, in whole, by the way of organization of the combs in the nest of the cell starter colony. It is obvious that the rate of acceptance of the grafted larvae into artificial queen cells from the queen-cells bar frame is primarily influenced by the organization technology, of specificity and type of combs introduced into the starter family (Table 2).

Table 2. Results of acceptance of grafted larvae into artificial queen cells and introduced into starter families, in profile on experimental batches

Experimental batches wih starter colonies	Number of larvae			Acceptance difference versus control,%	The criterion of certainty of difference (t_d)
	grafted	accepted	% acceptance		
Series I (24.04 – 03.05)					
Batch I (control)	60 ± 0.0	21.0 ± 1.0	35.0 ± 1.7	-	-
Batch II	60 ± 0.0	54.2 ± 0.7	90.3 ± 1.1	+55.3	27.3***
Batch III	60 ± 0.0	24.8 ± 1.7	41.3 ± 2.8	+6.3	1.9*
Batch IV	60 ± 0.0	50.2 ± 0.6	83.7 ± 1.0	+48.7	24.7***
Series II (04.05 – 13.05)					
Batch I (control)	65 ± 0.0	27.8 ± 1.1	42.8 ± 1.8	-	-
Batch II	65 ± 0.0	59.4 ± 0.7	91.4 ± 1.0	+48.6	23.6***
Batch III	65 ± 0.0	27.0 ± 0.9	41.5 ± 1.5	-1.3	0.5
Batch IV	65 ± 0.0	55.0 ± 1.6	84.6 ± 2.4	+41.8	13.9***
Series III (14.05 – 23.05)					
Batch I (control)	55 ± 0.0	22.2 ± 1.6	40.4 ± 2.8	-	-
Batch II	55 ± 0.0	51.6 ± 0.7	93.8 ± 1.6	+53.4	16.6***
Batch III	55 ± 0.0	22.0 ± 0.7	40.0 ± 1.3	-0.4	0.1
Batch IV	55 ± 0.0	44.4 ± 1.2	80.7 ± 2.1	+40.3	11.5***
Total in all series					
Batch I (control)	180 ± 0.0	66.0 ± 3.7	36.7 ± 2.1	-	-
Batch II	180 ± 0.0	165.2 ± 0.8	91.8 ± 0.5	+55.1	25.5***
Batch III	180 ± 0.0	73.8 ± 2.0	41.0 ± 1.1	+4.3	1.8*
Batch IV	180 ± 0.0	149.6 ± 1.5	83.1 ± 0.8	+46.4	20.7***

*Remark: * - P < 0.1; *** - P < 0.001.*

The obtained data denotes the fact that, in all growth series, the highest larval acceptance rate was recorded in experimental batches II and IV, where, in addition to the two food combs (honey+bee bread) and the queen cell bar frame there were added 2 brood combs of all ages inclusiv with unsealed larvae. At the same time, in all growth series, the lowest rate of larval acceptance was recorded in the I and III groups, in which besides the 2 food combs (honey + bee bread) and the queen-cells bar frame, no brood combs were introduced or only sealed brood comb have been added.

Thus, in the first series of rearing of grafted larvae into the starter families of batch II, in which two brood combs of all ages were introduced, of the 60 larvae introduced into each family, several larvae were accepted, comparative with families in the control group, averaging 33.2 larvae. In this case, the rate of larval acceptance was biger than 2.6 times (t_d = 27.3; P<0.001). Also in the starter families of batc IV, where 2 unsealed brood combs were introduced, the larvae acceptance rate was higher compared to that of the control group families, 2.4 times (t_d = 24.7; P<0.001). At the same time, in the starter families of batch III, in which in addition 2 sealed brood combs were introduced, the acceptance rate of the larvae tended to be higher compared to the control group by 6.3% (t_d=1.9; P<0.1).

In the second and third series the same regularity of the larvae acceptance in the starter bee families of different batches was recorded. Thus, the starter families of batch II, in which 2 brood combs of all ages were introduced, both in the second and third series, had a higher larval acceptance rate, compared to the control

group of 2.1 and 2.3 times, respectively (t_d=23.6 and 16.6; P<0.001). In starter colonies of batch IV, in which 2 unsealed brood combs were introduced, the larvae acceptance rate was higher than in the families of the control group, both in the second series of rearing, and in the third, 1.98 and 1.99 times, respectively (t_d=13.9 and 11.5; P<0.001).

In total, on all rearing series, the larval acceptance rate in the starter families of batch II, in which 2 brood combs of all ages were additionally introduced, was quite high, averaging 91.8±0.5% being higher than in the control group families (36.7±2.1%), by 2.5 times (t_d=25.5; P<0.001). In bee families of batch IV, where 2 unsealed brood combs were added, the larval acceptance rate was also high, on average on all growth series 83.1±0.8%, being higher than in the control batch on 2.3 times (t_d=20,7; P<0,001). At the same time, in the starter families of batch III, where 2 sealed brood combs were added, the larval acceptance rate had on average on all the rearing series only a tendency to be higher compared to the control group with 4.3% (td = 1.8; P<0.1).

Thus, generalizing data on larval acceptance rate in cell starter families, depending on the tipe of combs organization in the nest, we can conclude that the introduction of the brood combs of all ages, as well as of the unsealed brood combs, leads to a significant increase of the larval acceptance rate, respectively to the increase of the efficiency of using the cell starter colonies. After us, the presence of all age brood, especially of the unsealed one, causes an instinctive attraction of young bees in the starter's nest, being activated by the brood care instinct of the nursing bees. In the absence of the brood, a big number of bees leave the starter nest. According to H. Ruttner (1980), the existence of brood in the starter colony, contributes to the increase of royal jelly amount, produced by nurses. According to Билаш Г. (1991), "the less unsealed brood have the family , the sooner it begins to build queen cells randomly, after the queen has been emerged the sooner it begins to build queen cells randomly, after the queen has been emerged". This author affirm that "beyond any doubt, the presence of the brood in the starter family, positively influences their food supply. The brood from the starter colony combats the phenomenon of bee desertion and maintains the nest's integrity. The nurse bees are concentrated on unsealed brood combs, that's why these combs are placed next to the queen-cells bar frame, so that the nursing bees can feed the queen larvae in abundance. In addition, the optimal air temperature can be maintained only in the presence of the brood in the bee nest". The last sentence of the quoted text seems to clarify the role of the sealed brood in favor of the larval acceptance rate in the cell starter colony. If it is clear that the unsealed brood stimulates the production of the royal jelly by the nurses, then it is concludent that the sealed brood attracts the bees to keep the optimum temperature in the nest. This explains why the highest rate of larval acceptance in our experiment was recorded in the starter colonies with brood combs of all ages. Although, the role of sealed brood in the starter family is lower compared to unsealed brood, yet this (influence) is often significant.

Thus, in the first series of rearing, the acceptance rate of the grafted larvae into the starter families of batch III, in which were introduced sealed brood combs, was higher compared to that of the control batches families by 6.3% (t_d=1.9; P<0.1). On average on all rearing series, the larvae acceptance rate in the starter families of group III, in which sealed brood combs were introduced, was higher, compared to that in the control group families by 4.3% (t_d=1.8; P<0.1).

The method of the combs organizing in the nest of the starter colony obviously influences the efficiency of queen rearing, expressed in the final results - the percentage (yield) of the raised queens from the total number of grafted and introduced for growth larvae in the starter families (Table 3).

The results demonstrates that in all rearing series the highest yield of emerged queens were recorded in the cell starter families of batches II and IV, in which 2 brood combs were added and the lowest yield was recorded in the starter families of batches I (control) and III, where no brood combs were introduced or, respectively, only sealed brood combs were introduced. We have found out that in the first series of queen rearing (25.04-03.05) the yield of obtained queens from the bee families of batch II, in which 2 brood combs of all ages were added,

was higher, compared to the one in the families of the control group, in which no brood combs were introduced, by 3.05 times (t_d=19.2; P<0.001). Also the same regularity was recorded in the second and third series of rearing.

Table 3. Final results of queen rearing from grafted larvae, introduced into the starter colonies, in profile on the experimental batches

Experimental batches wih starter colonies	The amount of larvae introduced into the starter family	Number of emerged queens	The yield of obtained queens, %	Difference of obtained queens from the control, %	The criterion of certainty of difference, (t_d)
Series I (25.04 – 03.05)					
Batch I (control)	60 ± 0.0	15.6 ± 0.9	26.0 ± 1.4	-	-
Batch II	60 ± 0.0	47.6 ± 1.4	79.3 ± 2.4	53.3	19.2***
Batch III	60 ± 0.0	20.0 ± 1.7	33.3 ± 2.8	7.3	2.3*
Batch IV	60 ± 0.0	47.2 ± 0.9	78.7 ± 1.4	52.7	26.6***
Series II (04.05 – 13.05)					
Batch I (control)	65 ± 0.0	21.0 ± 1.8	32.3 ± 2.7	-	-
Batch II	65 ± 0.0	55.4 ± 0.7	85.2 ± 1.0	52.9	18.3***
Batch III	65 ± 0.0	22.6 ± 0.7	34.8 ± 1.1	2.5	0.8
Batch IV	65 ± 0.0	51.8 ± 1.9	79.7 ± 2.1	47.4	13.8***
Series III (14.05 – 23.05)					
Batch I (control)	55 ± 0.0	15.2 ± 1.0	27.6 ± 1.8	-	-
Batch II	55 ± 0.0	47.6 ± 0.9	86.5 ± 1.6	58.9	24.4***
Batch III	55 ± 0.0	18.8 ± 0.7	34.2 ± 1.3	6.6	3.0**
Batch IV	55 ± 0.0	42.2 ± 1.1	76.7 ± 2.1	49.1	17.8***
Total in all series					
Batch I (control)	180 ± 0.0	51.8 ± 2.1	28.8 ± 1.2	-	-
Batch II	180 ± 0.0	150.6 ± 2.7	83.7 ± 1.5	54.9	28.6***
Batch III	180 ± 0.0	61.4 ± 2.4	34.1 ± 1.3	5.3	3.0**
Batch IV	180 ± 0.0	141.2 ± 1.9	78.4 ± 1.1	49.6	30.6***

*Remark: * - P < 0.05; ** - P < 0.01; *** - P < 0.001.*

The yield of obtained queens in batch II, in which 2 brood combs of all ages were introduced, was higher than in the families of the control group, where no brood combs were introduced, on 2.64 and 3.13 times, respectively (t_d=18.3 and 24.4; P<0.001).

High efficiency, with an increased degree of significance according to the Student criterion, of the final results of the queens rearing, was also noted in the bee families of batch IV. Thus, in the first series of rearing, the yield of obtained queens in bee colonies of batch IV, in which two unsealed brood combs were added, was higher compared to that in the families of the control group, in which no brood combs were introduced, by 3.03 times (t_d=26.6; P<0.001). Also the same regularity was manifested in the second and third series of growth. The yield of queens rearing in the starter colonies of batch IV, in which two unsealed brood combs were added, was higher than in the families of the control group, in which no brood combs was added, of 2.47 and 2.78 times, respectively (t_d=13.8 and 17.8; P<0.001).

A much lesser tendency to increase the efficiency of rearing of queens, depending on the way of combs organizatin in starter families, also was recorded in the families of batch III. Thus, in the first (24.04-03.05) and third (15.05-23.05) rearing series, the yield of obtained queens from the starter colonies of batch III, in which additionaly 2 combs of brood were added, was significantly more higher than in the families of the control group, where no brood were introduced, with 7.3% and 6.6%, respectively (t_d=2.3 and 3.0; P<0.05 and P<0.01).

On average, on all rearing series, the yield of obtained queens in starter families of batch II, in which two brood combs of all ages were introduced, was higher than in the families of

the control group, in which there were no brood combs introduced, by 2.91 (t_d=28.6; P<0.001). The yield of queen rearing in the starter colonies of batch IV, in which two unsealed brood combs were added, was higher than in the families of the control group where no brood combs were introduced, on average by 2.72 times (t_d=30.6; P<0.001). Similarly, in the bee families of batch III, in which 2 brood combs were introduced in addition, the queen rearing yield was significantly higher than in the families of the control group, in which they were not introduced combs with brood, by 5.3% (t_d= 3.0; P<0.01).

Finally, generalizing the results of experimental testing of different ways of organization of the combs in the nest of cell starter colonies, we can say that adding of the combs with brood in starter families has a beneficial effect on the ultimate efficiency of directed (artificial) queens rearing.

CONCLUSIONS

The presence of the brood in the combs introduced into the cell starter families has a beneficial effect on the final efficiency on the directed queens rearing.

The introduction into the cell starter colonies of brood combs of all ages contributes to the obvious increase of the larval acceptance level of 2.5 times (t_d=25.5; P<0.001) and of the yield of obtained queens by 2.9 times (t_d=28.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced.

The introduction into the cell starter colonies of unsealed brood combs contributes to the obvious increase of the larval acceptance level of 2.3 times (t_d=20.7; P<0.001) and of the yield of obtained queens by 2.7 times (t_d=30.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced.

The introduction into the cell starter colonies only of the sealed brood combs contributes to no significant growth of the larval acceptance level by 4.3% (t_d=1.8; P<0.1) and of the yield of obtained queens by 5.3% (t_d=3.0; P<0.01), compared to the colonies in the control batch, where no brood combs were introduced.

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