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SOME FACTORS AFFECTING THE QUALITY OF ARTIFICIALLY REARED *Apis mellifera* L. QUEENS WITHIN HONEYBEE NURSING COLONIES

Abd Al-Fattah, M.A.[*]; E. I. Haggag[**] and Nora A. Mohamed[**]

*** Dept. Econ. Ent. & pesticides, Fac. Agric., Cairo Univ., Giza , Egypt**

**** Beekeeping Division, Plant Prot. Res. Inst., Ministry of Agriculture.**

ABSTRACT

This study was carried out to investigate the quantity (as % of accepted larvae, sealed queen cells and emerged queens) and quality (as weight of queen at emergence) of queen honeybees as affected by some environmental factors related to rearing process during two successive years, (2004 – 2005) at Giza region, Egypt. Significant increase in the percentages of acceptance, sealed queen cells and emerged queens as well as weight of queens at emergence were obtained when the empty artificial queen cups exposed 24 h. for rearing colonies before used in grafting than unexposed ones. Cups of grafted larvae which affixed to the 3rd and 2nd bar ′ s locations within grafting frame produced more frequently queens with heavy weight than those resulted on the 1st or 4th bar ′ s locations. Better numerous virgin queens could be successfully reared during summer and spring seasons than those occurred during autumn. The lowest percentage of queen emergence with lightest weight was recorded during winter season.

INTRODUCTION

It is known that honeybee rear queens naturally in three situations: the first when colony preparing for swarming, the second through supersedure when queen is old and weak and the third during emergency when the queen has been accidentally killed or lost. The number, shape and site of the constructed queen cells in each case is discriminated by specific characteristics, (Cale *et. al*., 1975).

Beekeepers have exploited the biology of queen rearing to rear queens on demand for breeding or production proposes and have found numerous factors that influence the proportion of potential queen larvae the bees actually rear into queens, (Laidlaw, 1979 and Morse, 1979). Furthermore, the factors involved in the selection of future queens are of interest in understanding the biology of honeybee reproduction and in themselves of importance for commercial queen rearing, (Breed *et. al*., 1985).

Among the most important factors is exposing the artificial queen cups to rearing colony for several hours before use in grafting. Some researchers reported that the drawing procedure of queen cups prior to use in grafting was significantly improved their acceptability, (Vuillaume, 1957 and Kither & Pickard, 1983). On the other hand, Johansson &Johansson (1978) claimed that the acclimatizing queen cups prior to transplantation by placing them in a colony for several hours is an unnecessary procedure.

The effect of location of a given larvae within honeybee queen rearing colony on the probability that it will be reared as a potential queens has be considered by various investigators, (Visscher, 1986 ;De-Grande, *et. al*., 1993 and Sharaf El- Din, *et, al*., 2000). The production and quality of

queens are, also, affected by rearing season, (Salem *et, al*., 1976, Ali, 1994, Sharaf El- Din *et, al*., 2000 and Abd Al-Fattah, *et, al*., 2003).

The objectives of the present study were to investigate the influence of exposing the artificial queen cups to rearing colony 24 h before their use in grafting, the level of bar (holding the grafted queen cups), within the grafting frame and rearing seasons on the rates of larvae acceptance, sealing queen cells, queen emergence and weight of newly emerged virgin queens.

MATERIALS AND METHODS

This study was undertaken at the apiary of Agricultural Experimental Station, Faculty of Agricultural, Cairo University at Giza during two successive years, (2004 & 2005). The effect of exposing (polishing) the empty artificial queen cups before use in grafting and the level at which the bar of grafted cups was hunge in the grafting frame within rearing colony during different seasons was evaluated through this work.

Preparing the starter- finisher rearing colonies:

Strong honeybee colonies of the local Carniolan F1, were weekly provided with a lot of young nurse bees, (by adding 2 capped worker brood combs just before emergence) and one pollen comb. This procedure occurs during 1-3 weeks according to season and prevailing environmental conditions. Daily feeding with about 66% sugar syrup for two weeks before and during the period of queen rearing was done, (Abd Al-Fattah, 2003).

24 hours before grafting, queen and all combs containing unsealed brood, except one, were removed from the experimental colonies and workers of each queenless colony were condensed (if a colony constructed from more than one chamber) in the lower brood chamber. Therefore, the combs of each selected queenless rearing colony were arranged as follows: two combs of food stores adhering the hive wall, one capped brood, one uncapped brood, one capped brood, two food stores and feeder .

The grafted frame was inserted between the unsealed and sealed brood. The accepted queen cells were checked 24 hours after introduction, then they left in their positions on the holding frame within each rearing colony to complete their development.

The mature queen cells (after 9-10 days of grafting), were removed and each queen cell was separated under ball screen cage on unripened honey comb in the same colony to emerge.

Three queen rearing colonies, were monthly prepared, as previously described, and used for raising 192 grafted queen cups (64 grafted cups /col.). Each plastic or wax cup in the following treatments was provided with a young worker larva about 24 h. old on a small droplet of 1:1 aqueous solution of royal jelly according to the commercial queen rearing technique (Laidlaw, 1979).

The treatments:-

1. Exposing empty queen cups before grafting to rearing colonies :

Thirty two of artificial queen cups (16 from each type) were leaving in a queenless rearing colony for 24 hours. The exposed (polished) cups were

experimentally used within 1-2 hours from removing. Another 32 plastic or wax cups (in equal no.) were grafted without exposing to colonies.

2. Level of bar carrying the grafted cups within rearing colonies:

The tested cups were alternatively arranged on a 4- bar grafting frame. Each bar holding 16 cups, so the total cups were 64 for each bar /colony. The 1st bar was directly hung under the top of grafting frame, the 2nd , 3rd and 4th bars were hung under the first bar at 5 cm. between each other. As soon as the for prepared queen cup bars were grafted, they inserted in the center of rearing colony and observed for acceptance one day later after grafting.

This work was repeated once each month during the experimental period and the obtained data were collected as means per seasons for statistical analysis. The following parameters were applied to evaluated the importance of the previous factors in the field of queen production: 1- Number. and percentages of accepted larvae, sealed queen cells and emerged queens. 2- Weight of newly emerged virgin queens (not more than 12 hours) using an electrical balance to the nearest 0.01 mg.

Statistical analysis :

The percentage of acceptance, sealed cells and emerged queens were firstly transferred to angular (are sine %) according to the rules of Gomez & Gomez (1976). The split split complete block design was followed for data analysis and the means were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Acceptance of queen cells in relation to the grafted cups, number of sealed queen cells, number of queen emergence as well as the weight of newly emerged virgin queens as being affected by exposing (polishing) empty artificial queen cups to rearing colony, the level bar within grafting frame and rearing season had been monthly studied during two successive years (2004- 2005).

1. Effect of exposing empty queen cups to rearing colonies before grafting:

It is clear from data in (Table 1) that exposing the artificial cups to the starter- finisher rearing colonies before grafting attained a significant increase in the larval accepted rate, (81.5%) than those grafted in unexposing cups, (74.0%).

The percentage of sealed queen cells was significantly higher using the exposed queen cups, (78.2%) than unexposed ones, (69.8%).

Similar positive influence was recorded for the emergence rate of virgin queen, where it was 76.1 % with exposed cups against 66.7 % when unexposed cups were used, (Table 1).

The weight of newly emerged virgin queens that emerged from queen cells previously exposed, as empty cups, to nursery colonies were significantly heavier, (176.0 mg./queen) than those obtained from cells not previously exposed, (171.1 mg./queen). There is nonsignificant difference between years of study for the exposing process as shown in Table (1).

Table(1):Effect of exposing (polishing) empty queen cell cups to rearing colonies before grafting on the percentage of accepted larvae, sealed queen cells and queen emergence as well as weight of emerged queens during seasons of two successive years (2004- 2005)

Season	First year 2004			Second year 2005			Mean/ two years		Mean/ season
	Polished cups	Unpolished cups	Av.	Polished cups	Unpolished cups	Av.	Polished cups	Unpolished cups	
Mean percentage of accepted larvae									
Spring	87.8	83.0	85.4	86.4	79.1	82.8	87.2	81.1	**84.2 a**
Summer	81.6	75.0	78.3	87.8	84.7	86.3	84.7	79.9	**82.3 a**
Autumn	77.8	68.1	73.0	77.1	70.5	73.8	77.5	69.3	**73.4 b**
Winter	77.8	65.6	71.7	75.4	65.6	70.5	76.6	65.6	**71.1 b**
Mean/ year	**81.3**	**72.9**	**77.1**	**81.7**	**75.0**	**78.4**	**81.5**	**74.0**	**77.8**
			a			**a**	**a**	**b**	
Mean percentage of sealed queen cells									
Spring	85.4	80.6	83	83.7	75.7	79.5	84.6	78.1	**81.3 a**
Summer	78.8	71.2	75.0	86.1	81.6	83.9	82.5	76.4	**79.4 a**
Autumn	75	65.7	70.4	74.0	66.0	70.0	74.5	65.9	**70.2 b**
Winter	75.7	63.2	69.5	67.0	54.2	60.6	71.4	58.7	**65.1 b**
Mean/ year	**78.7**	**70.2**	**74.5**	**77.7**	**69.4**	**73.5**	**78.2**	**69.8**	**74.0**
			a			**a**	**a**	**b**	
Mean percentage of emerged queens									
Spring	82.3	77.8	80.1	81.6	73.3	77.5	82.0	75.6	**78.8 a**
Summer	77.8	68.8	73.3	85.8	79.9	82.8	81.8	74.4	**78.1a**
Autumn	71.9	61.5	66.7	72.3	63.2	67.8	72.1	62.4	**67.3 b**
Winter	73.3	59.4	66.4	63.5	49.7	56.6	68.4	54.6	**61.5 b**
Mean/ year	**76.3**	**66.9**	**71.6**	**75.8**	**66.5**	**71.2**	**76.1**	**66.7**	**71.4**
			a			**a**	**a**	**b**	
Mean weight of emerged virgin queen (mg)									
Spring	177.5	173.2	175.4	177.9	173.5	175.7	177.7	173.4	**175.6 b**
Summer	181.7	177.1	179.4	181.8	174.1	178.0	181.8	175.6	**178.7 a**
Autumn	173.9	170.3	172.1	173.7	169.1	171.4	173.8	169.7	**171.8 c**
Winter	167.9	163.1	165.5	173.5	168.3	170.9	170.7	165.7	**168.2 d**
Mean/ year	**175.3**	**170.9**	**173.1**	**176.7**	**171.4**	**174**	**176**	**171.1**	**173.6**
			a			**a**	**a**	**b**	

Mean in the same column or row followed by the same letters don ذt differ significantly according to Duncan ذ s Multiple Range Test at level 5. %

These findings were agreed with the results obtained by Kither & Pickard, (1983) and Abd Al-Fattah, (1996). They proved that the exposing and drawing procedure of the artificial queen cups significantly improved the acceptability of these cups by bees when used in grafting process. This effect may be due to an acceptance promoting substance from nursing workers or removing any acceptance inhibiting substance, (Kither & Pickard, 1983). Besides, Vuillaume, (1957) and Bobrzecki & Prabucki, (1975) proved that worker bees modified both cell opening and length of the new artificial wax cups through drawing process to the suitable form during time of exposure.

2. Effect of cell bar level within grafting frame:

Results in Table (2) reveal that the acceptance percentages of the grafted larvae on the second (84.5%) and third (84.7%) bars within grafting frame were significantly higher than the other bar levels. On the other hand,

there was significant increase for the accepting rate of the grafted larvae on the first bar (75.8%) than those on the fourth ones, (66.1%).

As shown in Table (2) the rate of sealed queen cells of the levels of second and third grafting bars were significantly preceding the others. The sealed queen cells rate of the first bar was higher than those of the fourth ones. The percentages of sealed queen cells on the 3rd , 2nd , 1st and 4th bar′ s levels were 82.5%, 82.3%, 71.1% and 60.3%, respectively.

The mean rates of emerged queens from cells which holded on the 2nd and 3rd bar levels were attained the first position when compared with the 1st and 4th bars. Those rates were 80.8% and 80.9%, 67.9% and 56.1%, respectively, (Table 2). Significant differences were found between emergence rate on the 1st bar, which occupied the second position, and those recorded on the 4th ones, came in third position.

Table(2): Effect of level at which the transplanted larval bar hung in the grafting frame on the percentage of accepted larvae,sealed queen cells and queen emergence as well as weight of emerged queens during seasons of two successive years,(2004-2005).

Season	First year 2004					Second year 2005					Mean / two years				Mean/ season
	Bar level within grafting frame					Bar level within grafting frame					Bar level within grafting frame				
	1st	2nd	3rd	4th	Av.	1st	2nd	3rd	4th	Av.	1st	2nd	3rd	4th	
	Mean percentage of accepted larvae														
Spring	81.3	89.6	90.3	80.6	85.5	79.2	88.2	91.6	72.2	82.8	80.3	88.9	91	76.4	**84.2 a**
Summer	79.2	81.2	78.5	74.3	78.3	83.4	88.2	91.7	81.9	86.3	81.3	84.7	85.1	78.1	**82.3 a**
Autumn	72.2	84.8	79.2	55.6	72.2	68.8	81.9	84.7	59.8	73.8	70.5	83.4	82.0	57.7	**73.4 b**
Winter	73.6	80.6	74.4	58.4	71.7	68.1	81.2	86.8	45.8	70.5	70.9	80.9	80.6	52.1	**71.1 b**
Mean	**76.6**	**84**	**80.6**	**67.2**	**77.1**	**74.9**	**84.9**	**88.7**	**64.9**	**78.4**	**75.8**	**84.5**	**84.7**	**66.1**	**77.8**
					a					**a**	**b**	**a**	**a**	**c**	
	Mean percentage of sealed queen cells														
Spring	78.5	87.5	88.9	76.4	82.8	75.7	86.8	90.3	65.3	79.5	77.1	87.2	89.6	70.9	**81.2 a**
Summer	74.4	79.8	76.4	70.8	75.4	81.3	86.8	91.0	76.4	83.9	77.9	83.3	83.7	73.6	**79.6 a**
Autumn	68.8	82.0	77.8	52.8	70.3	64.6	79.2	82.7	53.5	70.0	66.7	80.6	80.3	53.2	**70.2 b**
Winter	69.5	81.3	73.7	54.2	69.7	55.6	75.0	79.2	32.7	60.6	62.6	78.1	76.4	43.4	**65.1b**
Mean	**72.8**	**82.6**	**79.2**	**63.5**	**74.5**	**69.3**	**82**	**85.8**	**57**	**73.5**	**71.1**	**82.3**	**82.5**	**60.3**	**74.0**
					a					**a**	**b**	**a**	**a**	**c**	
	Mean percentage of emerged virgin queens														
Spring	75.0	86.2	87.5	71.6	80.1	72.3	86.1	90.3	61.2	77.5	73.7	86.2	88.9	66.4	**78.8 a**
Summer	72.3	79.2	75.0	67.4	73.5	79.9	86.8	90.3	74.3	82.5	76.1	83.0	82.7	70.9	**78.2 a**
Autumn	66.0	79.2	73.6	48.0	66.7	61.9	77.8	82.0	49.3	67.7	63.9	78.5	77.8	48.7	**67.2 b**
Winter	66.0	78.5	70.8	50.0	66.3	50.0	72.3	77.8	26.4	56.6	58.0	75.4	74.3	38.2	**61.5 b**
Mean	**69.8**	**80.8**	**76.7**	**59.2**	**71.6**	**66.0**	**80.8**	**85.1**	**52.8**	**71.2**	**67.9**	**80.8**	**80.9**	**56.1**	**71.4**
					a					**a**	**b**	**a**	**a**	**c**	
	Mean weight of newly emerged virgin queens (mg)														
Spring	174.8	176.2	176.5	174.0	175.4	175.3	176.6	176.7	174.1	175.7	175.0	176.4	176.6	174.0	**175.6 b**
Summer	178.7	180.4	180.6	177.9	179.4	178.5	177.3	179.2	176.8	178.0	178.6	178.9	179.9	177.4	**178.7 a**
Autumn	171.5	172.8	173.2	170.9	172.1	170.4	172.9	172.9	169.2	171.4	171.0	172.9	173.1	170.1	**171.8 c**
Winter	163.9	167.3	168	163.1	165.5	169.9	172.5	172.8	168.4	170.9	166.9	169.9	170.4	165.8	**168.2 d**
Mean	**172.2**	**174.2**	**174.6**	**171.5**	**173.1**	**173.5**	**174.8**	**175.4**	**172.1**	**174.0**	**172.9**	**174.5**	**175.0**	**171.8**	**173.6**
					a					**a**	**b**	**a**	**a**	**c**	

Mean in the same column or row followed by the same letters don′ t differ significantly according to Duncan ′ s Multiple Range Test at level 5 % .

The weight of newly emerged queens was clearly affected by their cell bar level within the grafting frame, (Table 2). A virgin queen emerged from cells of the 2nd and 3rd bar′ s levels were heavier in their weight than those emerged from cells of the others. Queen cells attached on the 1st bar took relatively more attention from workers where the emerged queens were heavier than those produced from queen cells of the 4th bar.

It is, also, noticed from data in Table (2) that there were non significant differences between the two years of investigation for all studied parameters.

It is obvious from the presented results that the level within grafting frame at which the bar of grafted cups was holded had an imported effect on the probability of queen being reared. The superiority was owned by the middle bars, (2nd & 3rd bar′ s level), then the top bar (1st bar's level) came next while the bottom bar (4th bar′ s level) was the latest. These finding were consider with those reported by many authors. Ali, (1994) collected the highest amounts of royal jelly from queen cells that attached on the 3rd bar within grafting frame. Macicka, (1985) and Sharaf El- Din, (2000) stated that the 3rd bar′ s level gave the best abdominal measurements and the heaviest body weight of the queen emerged from their adhesive queen cells.

On the other hand, temperature and humidity are more constant in the brood nest center than any other part within the colony, (Free & Spencer-Booth, 1959; Kronenberg & Heller, 1982 and Abd Al- Fattah, 1983). Therefore the brood rearing condition may have better away from the edges of hive. However, the top row of the grafted queen cups, (1st bar′ s level), might be receive more warm air than the lowest row, (4th bar′ s level) which might be more subject to cooling, (Visscher, 1986).

3. Effect of rearing season:

Production of honeybee queens was significantly affected by prevailing conditions throughout different seasons of the two studies years, (2004-2005).

Data presented in Tables (1&2) revealed significant differences in the mean rate of accepted larvae between both spring, (84.2%) and summer, (82.3%) from one side and both autumn, (73.4%) and winter, (71.1%) from the other ones.

Likewise, the percentages of sealed queen cells and emerged queens were followed the same trend during different rearing seasons. The corresponding figures during spring, summer, autumn and winter were 81.3 %, 79.4%, 70.2% and 65.1% for sealed queen cells and were 78.8%, 78.1%, 67.3% and 61.5% for emerged queens, respectively, (Table 1&2).

The variation in body weight of newly emerged virgin queens was clear between various seasons. The heaviest queens (178.7 mg.) were produced during summer season. Weight of virgin queens that emerged in spring was(175.6 mg.) significantly less than those resulted in summer season. The mean weight of queens that obtained during autumn was, (171.8 mg.) arranged in the third position. The lightest weight value of emerged queens was (168.2 mg.) registered during winter season as shown in Table (1&2).

It is obvious that the better queen parameters were recorded during active season, (spring & summer) due to the prevailing suitable

environmental conditions. These conditions included both weather factors and available food source, which positively reflected on the living conditions within the colony. The reaction of colony was appeared in the increasing of accepted larvae, sealed queen cells and emerged queens. Nasr, (1976) under the same circumstances, reported that the best time for queen rearing was from March to September. Besides, the nurse workers, during active season, produce and provide extra amounts of royal jelly to queen larvae. Therefore, heavy queens were obtained.

This conclusion was in agreement with the findings of Ali, (1994) who recorded the highest acceptance rate and quantity of royal jelly during summer, than spring and autumn seasons, while the lowest amounts were in winter season. Many authors in the same and other areas were also, agreed with this conclusion (Salem *et. al*., 1976 ; Eid, *et. al*., 1980 ; El- Sarrag & Nagia, 1985; Sharaf El-Din, 2000) and Abd Al- Fattah, *et. al*., 2003).

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بعض العوامل المؤثرة على جودة ملكات نحل العسل المرباه صناعياً داخل الطوائف الحاضنة

محمد عبد الوهاب عبد الفتاح* ، السيد إبراهيم حجاج ** و نورا عبد الهادي محمد**
*** قسم الحشرات الاقتصادية و المبيدات – كلية الزراعة – جامعة القاهرة**
**** قسم بحوث النحل – مركز البحوث الزراعية – وزارة الزراعة**

أجري هذا البحث لتقييم تأثير تقديم الكؤوس الصناعية الفارغة لمدة ٢٤ ساعة لطوائف التربية وكذلك مستوي الشريحة الحاملة للكؤوس المطعومة داخل إطار التربية على كل من نسبة القبول ونسبة غلق البيوت الملكية ومعدل خروج الملكات العذارى منها و كذلك أوزان الملكات العذارى الناتجة خلال المواسم المختلفة لسنوات ٢٠٠٤ ، ٢٠٠٥ .

و أظهرت الدراسة النتائج التالية :

أولاً : تأثير تقديم الكؤوس الفارغة:

بلغت نسبة القبول في الكؤوس المعرضة ٨١,٥ % مقابل ٧٤,٠% في الكؤوس غير المعرضة وكانت نسبة الغلق للبيوت الملكية ٧٨,٣% و ٦٩,٨% بينما بلغت نسبة خروج الملكات الجديدة ٧٦,١% و ٦٦,٧% للعاملين على الترتيب . وبلغت أوزان الملكات العذارى الناتجة ١٧٦,٠ ملجم و ١٧١,١ ملجم للعاملين على الترتيب .

ثانياً : تأثير مستوى السدابة :

ارتفعت كمية الملكات الناتجة وجودة الملكات المرباة على السدابتين الثانية و الثالثة ثم السدابة الأولى و أخيرا السدابة الرابعة حيث كانت نسبة القبول ٨٤,٥% ، ٨٤,٧% ، ٧٥,٨% و ٦٦,١% على الترتيب . أما نسبة الغلق فكانت ٨٢,٣% ، ٨٢,٥% ، ٧١,١% ، ٦٠,٣% على الترتيب . وبلغت نسبة خروج الملكات الجديدة ٨٠,٨% ، ٨٠,٩% ، ٦٧,٩% ، ٥٦,١% على التوالي . أما أوزان الملكات الناتجة فكانت١٧٤,٥ ، ١٧٥,٠ ، ١٧٢,٩ ، ١٧١,٨ ملجم على التوالي .

ثالثاً : تأثير مواسم التربية :

ازدادت نسبة القبول وغلق البيوت الملكية و خروج الملكات الجديدة خلال فصلي الربيع و الصيف ثم الخريف و أخيراً فصل الشتاء خلال عامي الدراسة . أما أوزان الملكات العذارى فكانت ١٧٨,٧ ملجم في الصيف، ١٧٥,٦ ملجم للربيع و ١٧١,٨ للخريف . أخيراً ١٦٨,٢ ملجم للشتاء . و لم نلاحظ أي فروق معنوية لأي صفة بين عامي الدراسة .