Effect of Virgin Queens Storage on Their Survival Rate, Attractiveness and Acceptance by the Honey Bee Colonies

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ABSTRACT

Virgin honeybee queens, were held in the centre of brood nest of both queenless and queenright strong colonies using two types of cages and fed by workers through a wire screen holes, emerging cage (EC) with two wire screen sides and Benton cage (BC) with one side of wire screen. Mean survival rate of stored queens in both cage types were not differ significantly. Storage virgin queens within queen-right colony was influenced reversely by the presence of colony laying queen as the survival rate was significantly lower (68.0%) than queens stored in queenless colonies (77.4%). The attractiveness of introduced virgin queens 30 days old to workers increased when the duration of queenlessness increase from 1 to 7 days and also, the acceptance percentage of them. The number of workers attracted by virgin queens was increased with the storage periods (3, 15 and 30days) as the lowest significant number was for the 3 days period, while there were no significant differences between the rest of periods. The younger and older queens were most significantly accepted than the intermediate ones. Workers in the 5 days queenless nuclei were more attracted and easily accepted the introduced virgin queens than those in similar queenless strong colonies.

INTRODUCTION

Recently, it is important to store a big number of honeybee queens in a good health to provide the different requirements of beekeepers as well as research purposes. The use of holding colonies is the most economic and efficient system of long-term queen storage to reduce the need for many extra colonies or using baby nuclei (Laidlaw & Eckert, 1962; Harp, 1967; Mackensen and Tucker 1970; Morse 1979; Ratia 2000; Siuda *et al.* 2011). Foti *et al.*, (1962) designed a small plastic cage to confine a queen with some workers. Poole *et al.,* (1973) described a simplified method for storage queens in which queens were placed in small plastic cups inverted over a gauze cage containing about one pound of workers. Other cages with different designs were tested for introducing virgin or mated queens in queenlees or queenright colonies, (Walton and Smith 1970; Szabo & Townsend, 1974, Levinsohn & Leneky, 1981; Shawer *et al.,* 1983; Wilde 2006 and Siuda *et al.* 2014). Many authors investigated a relationship between the age of honeybee queen and their attractiveness using several colonies for storing queens and supplying workers (Yadava& Smith, 1971 and Szabo, 1974 a &1977). Queens are most readily accepted by small queenless colonies, particularly during a nectar flow (Langetroth, 1890 and Morse, 1979).

Szabo, (1977) and Shawer, (1981) found that increasing the orphan degree of workers (queenlessness) lead to decrease the number of rejected queens which introduced to them.

The present paper investigates the survival rate of virgin queens solitary confined in two types of common cages and stored in queenless or queenright colonies. Also, the attractiveness, and acceptance of stored queens of different ages to workers in different colony strength and the effect of queenlessness duration were also studied.

MATERIAL AND METHODS

The experiments were conducted during the spring and early summer (March to June) of 2014 at apiary of Agricultural Faculty, Cairo University. The first hybrid virgin Carniolan queens were reared according to Doolittle method (1889) with defined larvae (1-2 days old). On emergence, each queen was caged individually without attendant workers or candy. These caged queens were held in the brood nest centre of the experimental colonies using a holding frame of emerging cages, (each carried 20 cages). All colonies were fed with 50% sugar syrup (w/w) 3 times before introducing queens, then the feeding was continued at 5 days intervals, except during the clover nectar flow. The following subjects were studied.

Experiment 1

The effect of storage cage type on the survival rate of the stored virgin queens in both queenless and queenright colonies was evaluated, the emerging cage (EC) and the Benton mailing cage (BC) (Levinsohn & Leneky, 1981; Shehata, 1982 and Gencer, 2003). Each emerging cage (5 x 5 x 2.5 cm.) was covered on both sides with wire screen mesh, while the Benton mailing cage (8×3×2 cm) had one side covered with the wire screen. The confined queens were fed by colony's workers through the wire screen holes (mesh 3 x 3 mm.). Eight colonies were used as a reservoirs divided into two groups (four colonies each). First group was queenless, while the second one was queenright and both of them had the same number of introduced virgin queens and cages types. Each colony contained 6-8 sealed brood combs and more than 14 combs covered with bees. Therefore, no supply with young bees or brood combs was done. The preparation of colonies started 2 days before the beginning of experiments by removing colony's queens (in case of queenless) or caging it (in case of queenright), then removing all uncapped brood to increase the acceptance of the stored queens and prevent rearing a new one (Gencer, 2003).

The holding frame which contained 20 queen cages (10 cages from each type) was inserted between two capped brood combs in the centre of the brood nest, in a way that where the covers of the cages were facing the bees (Johansson and Johansson, 1971; Szabo, 1974 b& 1977). After one week of inserting the stored queens in queenright colonies, the colony's queen was released in the colony for egg laying and it was observed after three days of releasing to ensure its acceptance, (Szabo, 1974 b).

Experiment 2

Effect of orphan's period (duration of queenlessness) of receiving colonies on the worker behavior toward introduced virgin queens 30 days old and their acceptance was studied. The tested periods of queenlessness were 1,3 and 7 days. Fifteen equal strength nuclei were prepared from five strong colonies (each contained 8 brood combs and 15 combs covered with workers) according to the following dates of queenlessness. The first group (5 nuclei) was on April 10, the second was on April

14 and the third was on April 16, then the tested virgin queens were introduced in April 17. The attractiveness was measured by counting the number of workers which attracted to each cage.

Experiment 3

Queen attractiveness and acceptance as affected by the following subjects. (1)colony strength, (2) age of stored virgin queens before releasing into different colony strength were tested. Sixty queenless colonies (5 days queenlessness), half of them were crowded colonies (10 combs each) in one brood chamber, use as strong and the rest half were nuclei (3 combs each) in swarm boxes. The stored queens at 24 h., 15 days and 30 days were tested in this trial.

Twenty virgin queens of each storage period were introduced into the strong and nuclei colonies (10 each) using screen mesh Benton cages. After 24 hours the attracted workers to each cage were counted, then queens were released within the colonies and were observed on the following three days.

Statistical analysis

Data obtained from the experiments were analyzed with the MSTAT-C (Version 2.10) computer program using analysis of variance (ANOVA) and the Duncan's Multiple Range Test to determine the differences between the obtained means. Percentages of survival were transformed to arc-sin for analyzing.

RESULTS AND DISCUSSION

Experiment 1

Data in Table 1 and Figure 1 showed the survival rates of virgin queens storied in both queenless and queenright colonies using two types of cages. Differences between mean survival rates of virgin queens in relation to cage type were not significant. Queens stored in BC had a higher insignificant survival rate (74.5%) than those stored in EC ones (71.1%). Meanwhile, the survival rate for queens stored in BC at any storage period was better than those stored in EC. Mean survival rate was influenced negatively with storage period, which was in agreement with Mohamed *et al.,* (2002), where they reported that only 66.7% of virgin queens survived after 3 weeks of storage in queenless colonies. Bigio *et al.,* (2012) reported that storing queens for 3 days resulted in 90 to 100% survival.

Table 1: Effect of storage period and cage type on stored virgin honeybee queens survival rate in both queenless and queenright colonies

Storage	Queenless colonies			Queenright colonies			Mean		Mean / period
	EC	BC	Mean	EC	BC	Mean	EC	BC	
	Survival rate	Survival rate	Survival rate	Survival rate	Survival rate	Survival rate	Survival rate	Survival rate	
24 h.	97.5	97.5	97.5	82.5	85.0	83.8	90.0	91.3	90.6
1 w.	77.5	92.5	83.0	77.5	82.5	80.0	77.5	87.5	82.5
2 w.	77.5	85.0	81.3	72.5	75.0	73.8	75.0	80.0	77.5
3 w.	75.0	77.5	76.3	67.5	70.0	68.8	71.3	73.8	72.5
4 w.	75.0	77.5	76.3	67.5	70.0	68.8	71.3	73.8	72.5
6 w.	72.5	70.0	71.3	62.5	67.5	65.0	67.5	68.8	68.1
8 w.	70.0	67.0	68.8	50.0	55.0	52.5	60.0	61.0	60.5
10 w.	62.5	67.0	65.0	50.0	52.5	51.3	56.3	59.8	58.0
Mean	75.9 a	79.3 a	77.4 A	66.3 b	69.7 b	68.0 B	71.1 (a)	74.5 (a)	72.8

EC Emerging cage, BC Benton cage
Means in the same row or (in brackets) followed by the same letter are not significant at 0.05 level according to Duncan's Multiple Range test.

The decline in EC may be due to the workers aggressiveness towards introduced queens and sting them through the two screen sides causing death, while in case of BC the queens can escape far towards the wooden side avoiding the sting apparatus. These results were confirmed with those obtained by Levinsohn and Lensky (1981) and Shawer *et al.,* (1983), where they found that storing queens in the individual case without direct worker access gave a better survival rate for a period ranged from 2.5 to 4.0 months with virgin queens.

Mean survival rate of queens in queenless colonies was significantly higher (77.4%) than survival rate of queens stored in queenright ones (68.0%). These results appeared that the presence of original mated free laying queen around caged virgin queens in the colony had more reversal effect on the survival rate of stored queens. This effect may be due to lack of workers attention towards stored queens, specially feeding behaviors.
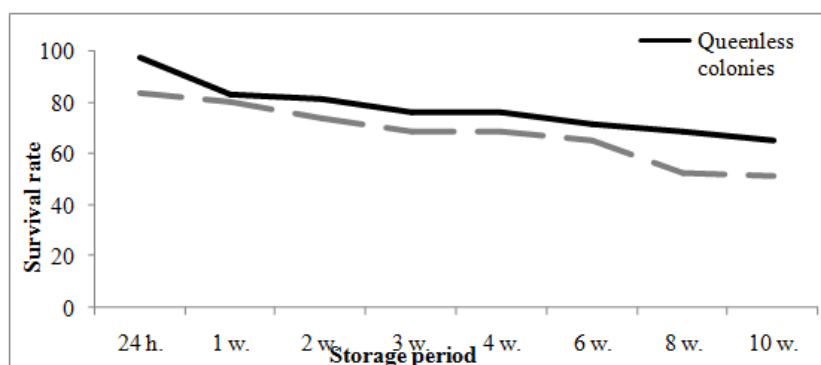

Fig. 1: Survival rate of virgin queens stored in both queenless and queenright colonies.

This conclusion is in agreement with that reported by Szabo (1974 a), who found that the movement of the colony laying queen around the stored virgin queens in the first brood chamber influenced the feeding of these queens which stored in that colony. He stated that, after 5 days of storage, the survival rate was ranged from 0.0% to 62.5%. So as, Levinsohn and Lensky (1981), they reported that In queenless or queenright compartments within queenright reservoir colonies, 69.0% to 91.0% of virgin queens survived for 5 months.

Experiment 2

This investigation obvious the effect of queenlessness period for small colonies (nuclei) on the number of workers attracted towards old virgin queens (30 days old) and on subsequent accepted them. Table 2 revealed that the high number of attracted workers was coincided with increasing the queenleasness period. These numbers were 46.2 ± 3.6, 28.6 ± 4.8 and 22.4 ± 4.7 bee / caged queen, when the periods of orphane were 7, 3 and 1 days respectively.

Table 2: Effect of orphan's period on the number of attracted workers and acceptance of virgin queens (30 days old).

orphan's period	Mean no. of attracted workers ± se	% acceptance after 24 h.
1 day	22.4 ± 4.7 C	80
3 days	28.6 ± 4.8 B	100
7 days	46.2 ± 3.6 A	100

Means marked with different letters are significantly different (P 0.05) according to Duncan's Multiple test.

Experiment 3

Results in Table 3 show the influence of different queens storage periods and the colony strength of queenless receiving colonies on the attractiveness (Fig. 2) and acceptance (Fig. 3) for these queens. It is clear that the lowest number of attracted workers recorded with queens 3 days old, (12.0 bee/caged queen). This attractiveness increased significantly with the increase of queen age, (24.5 and 30.7 bee/caged queen, for queens aged 15 and 30 days, respectively).

Table 3: Effect of queens storage periods and queenless colony strength on their attractiveness and acceptance by workers

Storage period	Strong Colonies		Nuclei		Mean	
	Mean no. Workers attracted	% acceptance	Mean no. workers attracted	% acceptance	No. workers attracted	% . acceptance
3 days	4.1 b	70.0 A	19.8 b	100 A	12.0 b	85.0 a
15 days	25.0 A	30.0 B	24.0 a	50.0 B	24.5 a	40.0 b
30 days	24.4 A	90.0 A	36.9 a	100.0 A	30.7 a	95.0 a
Mean ± se	17.8 ± 6.9 B	63.3 ± 19.6 (b)	26.9 ± 8.9 A	83.3 ± 18.5 (a)	22.4	70.4

Means in the same column marked with different letters are significantly different (P 0.01) according to Duncan's Multiple test.
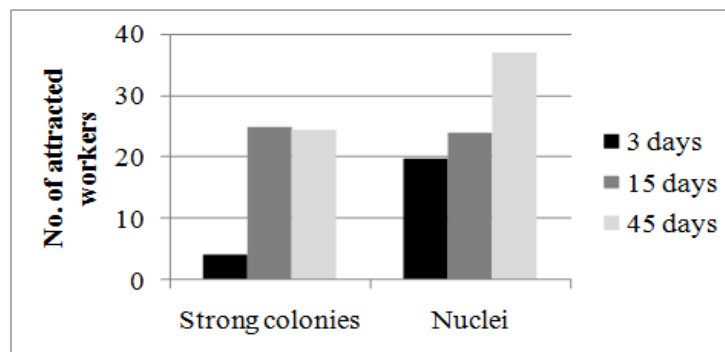

Fig 2: Effect of queenless colony strength on number of attracted workers towards virgin queens stored for various periods.
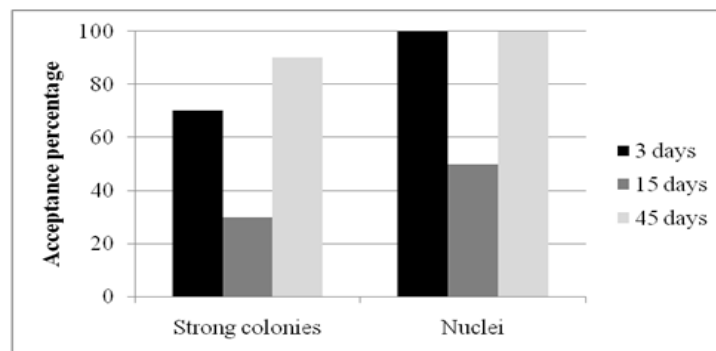

Fig. 3: Effect of queenless colony strength on acceptance percentage of virgin queens stored for various periods.

The younger and older virgin queens were more accepted (85.0% and 95.0%, respectively) than the intermediate ones (40.0%) and the differences between them were highly significant.

These results are agreement with those noticed by several authors (Yadava &

smith, 1971, Szabo, 1974 a and Shawer, 1981), where they found that queen attractiveness increased with age. Szabo, (1974 a) observed that the young (7 days) and old (35 days) queens, which were the least and the most attractive queens, were accepted more readily than the queens 14-28 days old which usually rejected. He suggested that the possible reason for this acceptance is that the former were not recognized as queens where the latter accepted as sufficiently similar to the colony queen of the workers.

Concerning the effect of colony strength on the queen attractiveness to workers, it is shown in Table 3 that the average number of attracted workers in nuclei was significantly greater (26.9 ± 8.9 bee/caged queen) than those recorded in strong colonies (17.8 ± 6.9 bee/caged queen). The acceptance percentage was, also, significantly higher in the nuclei, (83.3 ± 18.5 %) than the strong colonies (63.3 ± 19.6%).

This may be attributed to that the age of most workers which present within nuclei was less than 21 days, and those young bees are much less aggressive than the older ones. Practical beekeepers have for a long time suspected this age-linked behavior of workers towards queens, and utilized, in queen introduction (Szabo & Townsed 1974 and Morse, 1979).

This conclusion is confirmed with the previous procedure which occurred by Siuda *et al.,* (2014) where they showed that 59% of queens stored in strong colonies survived, while 77% of them survived when stored in 3-comb nuclei.

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ARABIC SUMMERY

تأثير تخزين الملكات العذارى على معدل بقائها، إنجذاب الشغالات لها ومعدل قبولها بواسطة طوائف نحل العسل

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تم إجراء هذا البحث في منحل كلية الزراعة – جامعة القاهرة خلال موسمي الربيع والصيف لعام 2014، وذلك لتحديد تأثير نوع قفص التخزين (قفص التحضين وقفص بنتون) على معدل البقاء للملكات العذارى المُخزنة داخل طوائف ذات ملكات وأخرى يتيمة، ودراسة تأثير عُمر الملكة، قوة الطائفة وطول فترة التيتم على قدرة الملكات على جذب الشغالات ومعدل قبول هذه الملكات. أولاً تم تخزين الملكات العذارى في نوعي الأقفاص داخل طوائف يتيمة وأخرى ذات ملكات وتبين عدم وجود فروق معنوية بين معدل البقاء في نوعي الأقفاص، في حين لوحظ أن معدل بقاء الملكات المُخزنة داخل الطوائف اليتيمة كان أعلى معنوياً (77.4%) عن معدل بقاء الملكات المَخزنة في الطوائف ذات الملكات (68%). ثانياً تم تخزين الملكات العذارى لمدة 30 يوم ثم إدخالها على نويات تختلف في طول فترة تيتيمها (1 و 3 و 7 أيام) وملاحظة عدد الشغالات المُنجذبة ومعدل القبول لهذه الملكات ، حيث وجد تفوق معنوي في عدد الشغالات المُنجذبة بزيادة فترة التيتيم لـ 7 أيام وكذلك معدل القبول (18,5 ± 46,2 و100%). ثالثاً تم تخزين الملكات لفترات مختلفة (3 و 15 و 30 يوم) ثم إدخالها على نويات وطوائف قوية وتحديد عدد الشغالات المُنجذبة ومعدل القبول في كل حالة. وجد أن عدد الشغالات المُنجذبة للملكات العذارى المُخزنة يزاد طردياً بزيادة مدة التخزين. معدل قبول الملكات المُخزنة لمدة 3 أيام و30 يوم كان متساوي معنوياً (85% و 95%) ومتفوق على معدل قبول الملكات المُخزنة لمدة 15 يوم (40%). كما أن عدد الشغالات المُنجذبة ومعدل قبول الملكات كان أعلى معنوياً في حالة النويات (8,9 ± 26,9 و 18,5 ± 83,3%) عن الطوائف القوية (6,9 ± 17,8 و19,6 ± 63,3%) على الترتيب.