EFFECT OF QUEEN CELL PREPARATION ON LARVAE ACCEPTANCE IN STARTER HONEYBEE COLONIES

UTJECAJ PRIPREME UMJETNIH MATIČNJAKA NA PRIHVAT PRESAĐENIH LIČINKI U STARTERIMA PČELINJIH ZAJEDNICA

KOVACIC, Marin & PUSKADIJA, Zlatko

Abstract: *The aim of experiment was to determine if there is difference of larvae acceptance after grafting one day old larvae in artificial wax queen cells that were for one hour prior to grafting in starter colonies for polishing (P) and artificial wax queen cells that weren't in contact with bees (C). Furthermore, 20 queens from each group after hatching were weighed and dissected to measure diameter of spermatheca. 24 hours after grafting average acceptance of larvae in group P and C was 85.3% (min-max: 78-89.8) and 76.6 % (min-max: 66-82.5) respectively. Polishing of artificial wax queen cells before grafting have significant effect (p<0.05) on acceptance of grafted larvae, but not on the size of spermatheca and weight of queen.*

Key words: *Apis mellifera, queen cell, grafting*

Sažetak: *Cilj istraživanja bio je utvrditi ima li razlike u uspjeha prihvata presađenih jednodnevnih ličinki u umjetne početke matičnjaka koji su bili u starteru sat vremena prije presađivanja (skupina P) i umjetnih početaka matičnjaka koji nisu bili u prethodnom doticaju s pčelama (skupina C). Osim toga kod 20 matica iz svake skupine izmjerena je težina matice i promjer spermateke. 24 sata nakon presađivanja skupina A imala je prosječan prihvat od 85,3% (min-max:78-89,8), dok je skupina B imala prosječan prihvat od 76,6% (min-max:66-82,5). Umetanje matičnjaka prije presađivanja u starter ima značajan utjecaj (p<0,05) na uspjeh presađivanja, ali ne na težinu izleženih matica i veličinu spermateke.*

Ključne riječi*: Apis mellifera, matičnjak, presađivanje*

Author's data: Marin, **Kovačić**, dipl.ing., University of J.J.Strossmayer in Osijek, Faculty of agriculture, komarin@pfos.hr; Zlatko, **Puškadija**, prof.dr.sc., University of J.J.Strossmayer in Osijek, Faculty of agriculture, zpuskadi@pfos.hr

1. Introduction

In honey bees (*Apis mellifera* L.), the quality of the queen undeniably affects the colony performance. Quality of the queen can be evaluated by characters of the queen live weight, weight and number of ovariole, size of the spermatheca, fecundity (number of eggs laid per day) and brood quality. All above mentioned characters are highly depending on the conditions when the queen is grown.
Commercial rearing of queens requires huge production of high quality queens [1]. Doolittle [2] was first who grafted worker larvae to produce queens, and since then several research was made about factors affecting the success of grafting: age of grafted larvae [9, 10, 11, 12], design and position of artificial queen cells [3, 13], priming queen cells with royal jelly before grafting [3, 4, 8], position of queen cell with transferred larvae in hive [5, 8, 13], feeding of queen rearing colony [11, 14]. The body weight of the queen is one of the first evaluations which breeders can make with emerged queen. Best evaluation of the queen weight is made inside first few hours after hatching because decline in the queen body weight is most rapid in first 36 hours [6].
Some of the basic factors for acceptance of grafted larvae by queen cell building colony are: strength of colony, food storage, number of grafted queen-cells, age of the worker bees, age of the grafted larvae, presence of queen and presence of open brood in rearing colonies [4, 7, 9]. Beside genetic origin of the queen, high quality queen cell is one of the most important steps in rearing biologically superior queens. With this research we wanted to investigate does preparing of artificial queen cells before grafting affects (1) the success of grafting, (2) the queen weight and (3) spermatheca size.

2. Materials and methods

Eight starter colonies for experiment were formed three days prior grafting. Each starter colony was prepared with shaken bees from 40 frames of brood (where most of the nurse bees are expected to be found [15]) and 5 frames of honey and pollen. One frame of young brood was present in starter colony when there were no queen cells, but before inserting grafted cells brood frame was removed so it is ensured that most of nursing bees will be on queen cells. Every three days each starter received 120 grafted cells and totally 4 repeated series were performed. After the second series of grafting, starters were refreshed with young bees. Artificial wax queen cells were used for grafting one day old larvae to get high quality queens [16].
Two groups of 4 starter colonies were formed. In the first group, one hour prior to grafting, queen cells were added for polishing (group P). After removal of the queen cell from colony for grafting, it was clearly evident that bees added some new wax on edges of the queen cell. Chinese grafting tool was used for grafting, so there was no need to prime queen cells with royal jelly since some of it is grafted together with the larvae. After grafting, polished queen cells were added back to group P starters, while

control group (group C) received grafted larvae in queen cells that were not in contact with bees before grafting. Upon receiving grafted material, each starter was fed with 1 litre of sugar syrup (50:50). After 24 hours, checking for accepted larvae was made. Total 40 randomly chosen queens (20 from each group) after hatching was stored in freezer on -18°C. Afterwards, the queens were weighed with electric balance to the nearest 0.01 mg and dissected for measuring diameter of spermatheca as described in [17]. Statistical analysis was made in Statistica 12 software.

3. Results and discussion

In total 3.840 larvae was grafted. The percentage of accepted queen cells in group P and C was 85.3% and 76.6% respectively which is significant difference ($t=-2.13$, $p<0.05$) (Table 1.). The results of acceptance rate are similar to Ebadi and Gary [4] and
Gancer et al. [11], who have 76,6% and 73,4% respectively.

Group	larval acceptance			min-max	Avarage
	accepted	rejected	total		
Control	1471	449	1920	66-82.5	76.6% (±14.6)
Polishing	1638	282	1920	78-89.8	85.3% (±7.3)

Table 1. Acceptance of grafted larvae

It is clearly evident that control starter colonies had much wider range of grafted larvae acceptance (Figure 1.) compared to polishing group. The results suggest that polishing of the queen cells before grafting is effective way to increase acceptance of grafted larvae. However, it is questionable whether it is worth to carry out this operation in major productions. Still, if problems occur with the rate of acceptance in normal queen cell production, polishing presents a promising way to increase it.

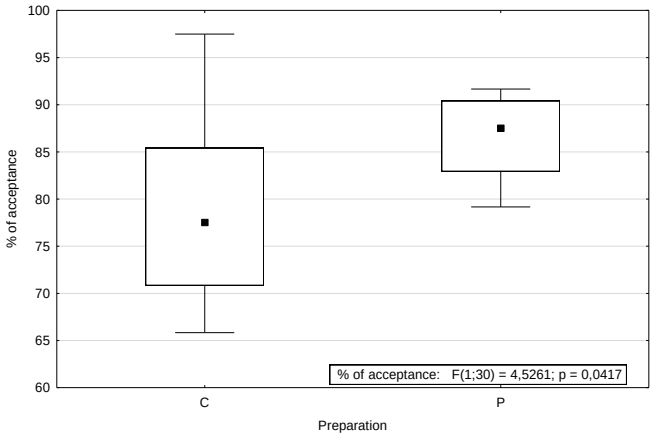

Figure 1. Acceptance of grafted larvae in C and P group

Preparation of queen cells had no significant effect on size of the spermatheca ($t=-1.36$, $p>0.05$) and weight of the queen after emergence ($t=-0.88$, $p>0.05$) between

groups. Though, a greater number of heavier queens were recorded in P group (Figure 2). Average spermatheca diameter in C and P group was 1,17 mm and 1,24 mm respectively, which is similar to results of Dražić et al. [18] where 1,10-1,19 mm spermatheca diameter was recorded. Positive correlation between queen weight and spermatheca size (r=0.45) was recorded. As expected, larger queens had larger diameter of spermatheca. Duly grafting of one day old larvae will provide a quality young queen that will be able to fulfil the potential development of the colony. Preparation of queen cell does not affect quality of the queen, rather enhances the success of accepting grafted larvae.

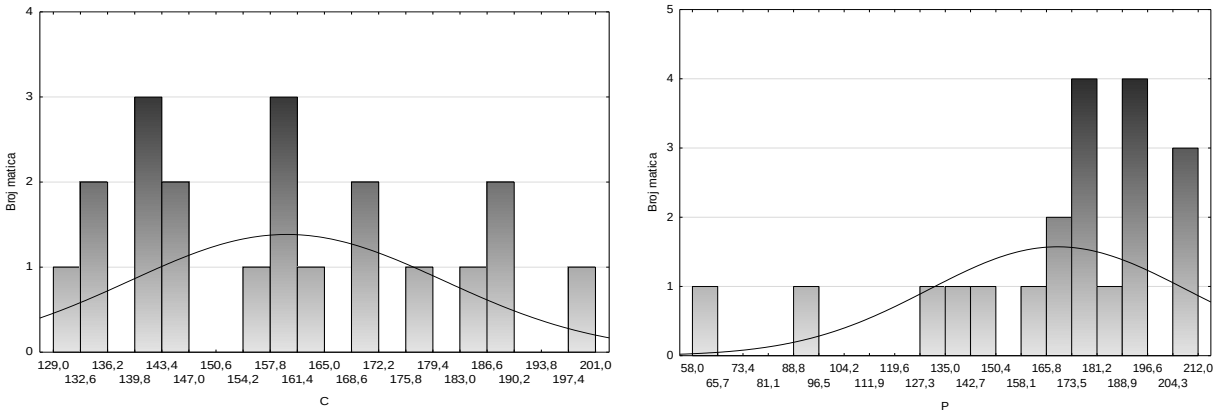

Figure 2. Weight of queens in mg 72 hours after hatching in control (left) and polishing group (right)

4. Conclusions

These results support the thesis that polishing the queen cells before grafting have significant effect on larvae acceptance and can help to increase acceptance of grafted larvae by rearing colonies if unfavourable conditions occurred. Preparation of queen cells before grafting does not affect the queen weight and the spermatheca size.

5. References

[1] Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezić, N., Kryger, P., Spivak, M., Uzunov, A., Wilde, J. (2013). Standard methods for rearing and selection of Apis mellifera queens. In: The *COLOSS BEEBOOK, Volume 1: standard methods for Apis mellifera research*. V, Dietemann; J. D. Ellis; P. Neumann (Eds), IBRA, ISBN: 0-86098-274-2, Treforest, UK

[2] Doolittle, G.M. (1889). *Scientific queen-rearing*, Thomas G. Newman & Son, 9781904846611, Chicago, Illinois,

[3] Kither, G.Y., Pickard, R.S. (1983). Increasing the acceptance of transplanted honeybee worker larvae by queen-cell starter colonies with the use of partially drawn artificial queen cups. *Journal of Apicultural Research*, 22(3): 175-183, ISSN: 0021-8839

[4] Ebadi, R., Gary, N.E. (1980). Acceptance by honeybee colonies of larvae in artificial queen cells. *Journal of Apicultural Research*, 19(2): 127-132, ISSN 1995-8994

[5] Visscher, K. (1986). Effect of location within the nest on acceptance of queen cells in honeybee colonies. *Journal of Apicultural Research*, 25(3): 154-157
[6] Skowronek, W., Bienkovska, M., Kruk, C. (2004). Changes in body weight of honeybee queens during their maturation. *Journal of Apicultural Science,* 48(2): 61-68
[7] Ruttner, F. (1983). *Queen rearing: biological basis and technical instruction*. Apimondia Publishing House, ISBN: 0955420830, Bucharest, Romania
[8]Pickard, R.S., Kither, G.Y. (1983). Acceptance of transplanted worker larvae by queen-cell starter colonies. *Journal of Apicultural Research*, 22(3): 169-174
[9] Woyke, J. (1971). Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *Journal of Apicultural Research*, 10(1): 45-55
[10] Gabka, J., Ochnio, M., Kaminski, Z., Madras-Majewska, B. (2011). Effect of age of eggs used for rearing honey bee queens on the number of received queen cells. *Journal of Apicultural Science*, 55(1), 47–53.
[11] Gancer, H. V., Shah, S. Q., Firatli, Ç. (2000). Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. *Pakistan Journal of Biological Sciences*, 3(8), 1319–1322.
[12] Mahbobi, A., Farshineh-Adl, M., Woyke, J., & Abbasi, S. (2012). Effects of the Age of Grafted Larvae and the Effects of Supplemental Feeding on Some Morphological Characteristics of Iranian Queen Honey Bees (*Apis mellifera meda* Skorikov, 1929). *Journal of Apicultural Science*, 56(1), 93–98.
[13] Al-Fattah, M. A. A., Mazeed, A. M., Al-Hady, N. A. (2011). Quality and quantity of honeybee queens as affected by the number and distribution of queen cells within queen rearing colonies. *Journal of Apicultural Science*, 55(2), 31–43.
[14] Sahinler, N., Gul, A., & Sahin, A. (2005). Vitamin E supplement in honey bee colonies to increase cell acceptance rate and royal jelly production. *Journal of Apicultural Research*, 44(2): 58–60.
[15] Pickard, R.S., Kither, G.Y. (1983). An evaluation of shaking and displacement methods for separating a sample of honeybees with large hypopharyngeal glands from the remainder of a colony. *Journal of Apicultural Research*, 22(2): 101-106
[16] Dedej, S., Hartfelder, K., Aumeier, P., Rosenkranz, P., Engels, W. (1998). Caste determination is a sequential process: effect of larval age at grafting on ovariole number, hind leg size and cephalic volatiles in the honey bee (Apis mellifera carnica). *Journal of Apicultural Research*, 37(3): 183–190.
[17] Carreck, N. L., Andree, M., Brent, C. S., Cox-Foster, D., Dade, H. a, Ellis, J. D., Hatjina, F., vanEnglesdorp, D. (2013). Standard methods for Apis mellifera anatomy and dissection. *Journal of Apicultural Research*, 52(4), 1–40.
[18] Dražić, M. M., Filipi, J., Caba, K., Pobran, L., Nikšić, M., Prđun, S., Bubalo, D., Kezić, N. (2015): Broj spermija u maticama Apis mellifera carnica iz hrvatskog uzgojnog programa. *Proceedings 50th Croatian and 10th International Symposium of Agriculture,* Opatija, 394-398.

Photo 027. Agreement / Sporazum