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INVESTIGATIONS OF HYGIENIC BEHAVIOUR AND DISEASE RESISTANCE IN ORGANIC BEEKEEPING OF TWO HONEYBEE ECOGEOGRAPHIC VARIETIES FROM SERBIA

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Hygienic behaviour of the carniolan honeybee (Apis mellifera carnica Pollm.) was investigated in 20 localities in Serbia (10 localities from the Machva region and 10 localities from the Rudnik region). The results revealed that the grey bees from Rudnik have a more expressed form of hygienic behavior, compared to the yellow bees from Machva. The obtained data indicate that colonies of both investigated honeybee varieties, yellow bees from Machva and grey bees from Rudnik, belong to a category of the so called "hygienic colonies", as the efficiency of elimination of damaged pupae amounted to 91,45% in Machva honeybees and 93,60% in Rudnik honeybees. Our results point to an indisputable relationship between hygienic behaviour and the strength of honeybee colonies, i.e. the potent colonies have more expressed hygienic behaviour. Both investigated honeybee varieties can be used for improving breeds selection and for organic beekeeping in Serbia, owing to the manifested hygienic behaviour and thence, resistance to some diseases (Varroa, American foulbrood, and especially Chalkbrood).

Key words: disease resistance, honeybee, hygienic behaviour, organic beekeeping, Serbia.

INTRODUCTION

The behaviour of honeybees, as of other animals, is dictated by some internal and external factors, being, at the same time, genetically predisposed in the same way as any other physical feature of the honeybee organism. On the basis of the results obtained so far it is believed that the honeybee´s hygienic behaviour is determined at two independent genetic loci. One of them controls the uncapping of the diseased brood (U-uncappe), whereas the other removes larvae and pupae (R-remove). The honeybees that are recessive homozygotes (uurr) clean the brood, eliminating from it the larvae and pupae infected with pathogens, whereas those whose genotype contains the alleles U and R, regardless of the type of combination, do not do it (Rothenbuhler, 1964a, b; Spivak & Gilliam, 1993).

Therefore, the hygienic behaviour of workers is inherited as a recessive property in the European honeybees, whereas the same behaviour is a dominant feature in the species *Apis cerana*.

The hygienic behaviour in honeybees, as well as in other organisms at a higher phylogenetic stadium, basically contributes to the maintenance of body hygiene, the hygiene of the progeny, the hygiene in hives, for shorter or longer periods after birth, and is, therefore, inseparable from their resistance to certain diseases. In particular, hygienic behavior has been shown to be a significant factor in resistance to Varroa, as well as American foulbrood, and especially Chalkbrood.

In this work an investigation of hygienic behaviour was carried out in order to determine the level of hygiene of two honeybee ecogeographic varieties from Serbia – yellow bees from the Machva region and grey bees from the Rudnik region. The question was posed: do intra- and interpopulation differences exist between the investigated ecogeographic varieties (ecogenotypes) with reference to this extremely important property of honeybee colonies, especially from the aspect of their resistance to some diseases, with a view of improving selection and rearing of quality queens. Breeding of resistant honeybee ecotypes, best adapted to living conditions, is necessary for organic beekeeping.

Organic beekeeping is a component of organic agriculture - an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. Organic agriculture is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony. The principal guidelines for organic production are to use materials and practices that enhance the ecological balance of natural systems and that integrate the parts of the farming system into an ecological whole. The primary goal of organic agriculture is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people. *(National Organic Standards Board - April 1995).* The primary goal of organic beekeeping is to find naturally (indigenous) disease-tolerant honeybee colonies, to propagate and maintain those honeybees in the absence of toxic chemicals, which implies the absence of toxic or contaminated substances in honey and other bee products (Le Guillou & Scharpe, 2001).

MATERIAL AND METHODS

The analysis of the hygienic behaviour of honeybee workers was conducted in 20 localities in Serbia (10 localities from the Machva region and 10 localities from the Rudnik region). At each locality we analysed 10 potent colonies with one-year old queens, 10 potent colonies with two-year or three-year old queens, 10 medium potent colonies and 10 weak colonies. i.e. 40 colonies at each locality. There were altogether 800 colonies in the two above mentioned regions (400 colonies in Machva and 400 colonies in Rudnik region). Hygienic behaviour of the chosen honeybee colonies was examined and evaluated according to the method of Taber (1982), as modified by Kefuss *et al.* (1996) and Stanimirović *et al.* (2001a): On a standardized frame from an LR (Langstroth-Root) or DB (Dadant-Blat) beehive, a diamond area of comb (5cm x 6cm) was marked for sampling with a tensile steel wire (Figure 1). Only one frame was sampled from

each analyzed beehive. The number of newly sealed brood cells in that area was counted and recorded. Cappings of newly sealed brood cells were punctured with a fine pin to kill the pupae beneath (Figure 2). Through the same hole pupae were pricked several times at different angles. Sampled frames with the sacrificed section of the brood were returned to the beehive. After 24 hours, the number of cells completely uncapped and cleaned out were counted and recorded. According to the criteria of Kefuss *et al.* (1996) as modified by Stanimirovic *et al.* (2001a, 2001b) colonies which cleaned more than 95% of the cells within 24 hours are considered superhygienic; colonies with an efficiency of pupae removal from the sampled area between 90% and 95% are considered hygienic, while nonhygienic colonies are those which cleaned less than 90% of the cells within 24 hours.

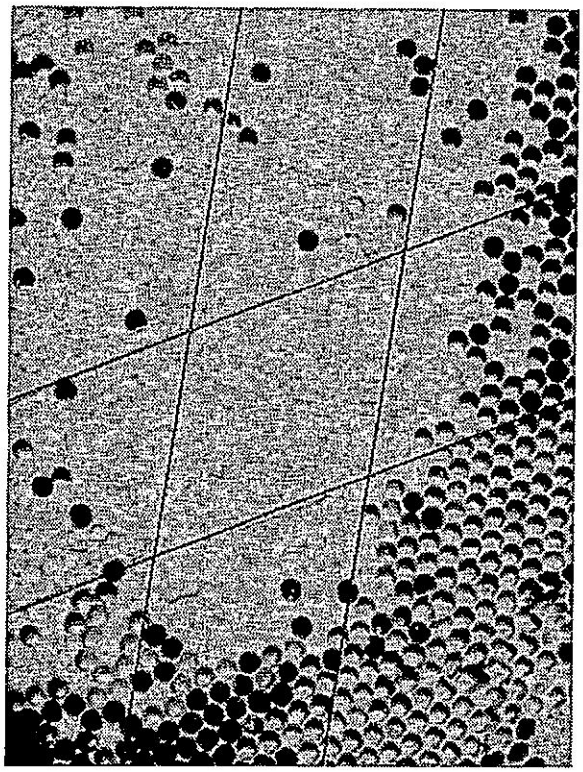

Figure 1. Diamond template of newly sealed brood prepared for pin-killed technique.

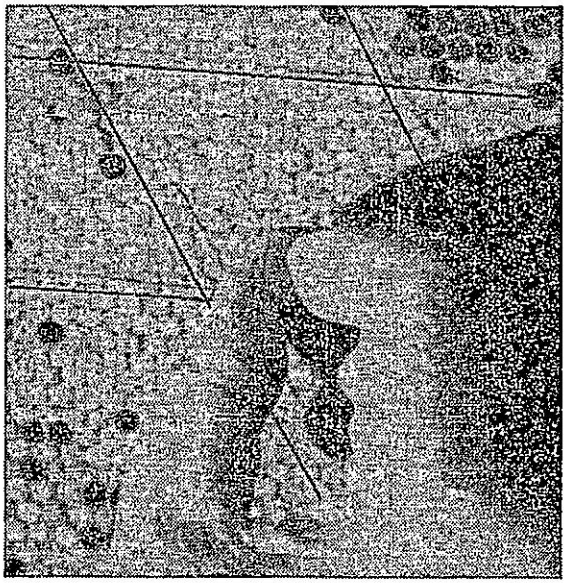

Figure 2. Pin-killed technique according to Kefuss *et al.* (1996), modified by Stanimirovic *et al.* (2001a)

RESULTS

In the Machva region, out of a total number of 200 investigated potent honeybee colonies, regardless of queen age, 180 colonies from 9 localities pertained to the category of superhygienic colonies (Figure 3 and 6). Meanwhile, in all investigated weak colonies and in 80 medium potent colonies, the efficiency of pupae removal from the sampled area was less that 90%, so those colonies were qualified as nonhygienic colonies (Figure 3). Potent colonies from Schtitar, regardless of queen age, and medium potent colonies from Schevarice and Majur were hygienic colonies.

In the Rudnik region, all investigated potent honeybee colonies, regardless of queen age (200 colonies from 10 localities), revealed superhygienic behaviour (Figure 4). The medium potent colonies from 8 localities were hygienic colonies, while medium potent colonies from G. Crnuca pertained to the category of superhygienic colonies. In medium potent colonies from G. Branetici and in all investigated weak colonies the efficiency of pupae removal from the sampled area was less that 90%, so those colonies were qualified as nonhygienic colonies (Figure 4).

The results of a comparison of percentage values of removed pupae between honeybee colonies from Rudnik and Machva regions are presented in Figure 5.

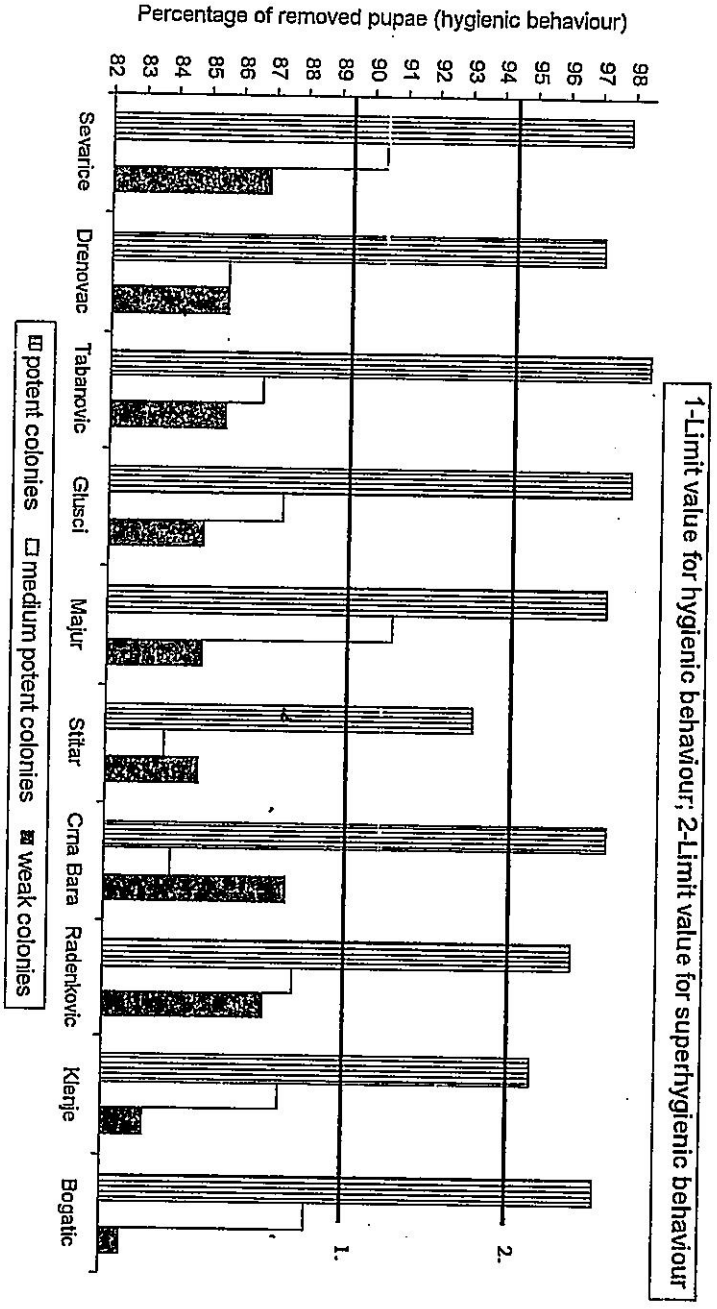

Figure 3. Comparison of honeybee hygienic behaviour at 10 different localities in the Machva region

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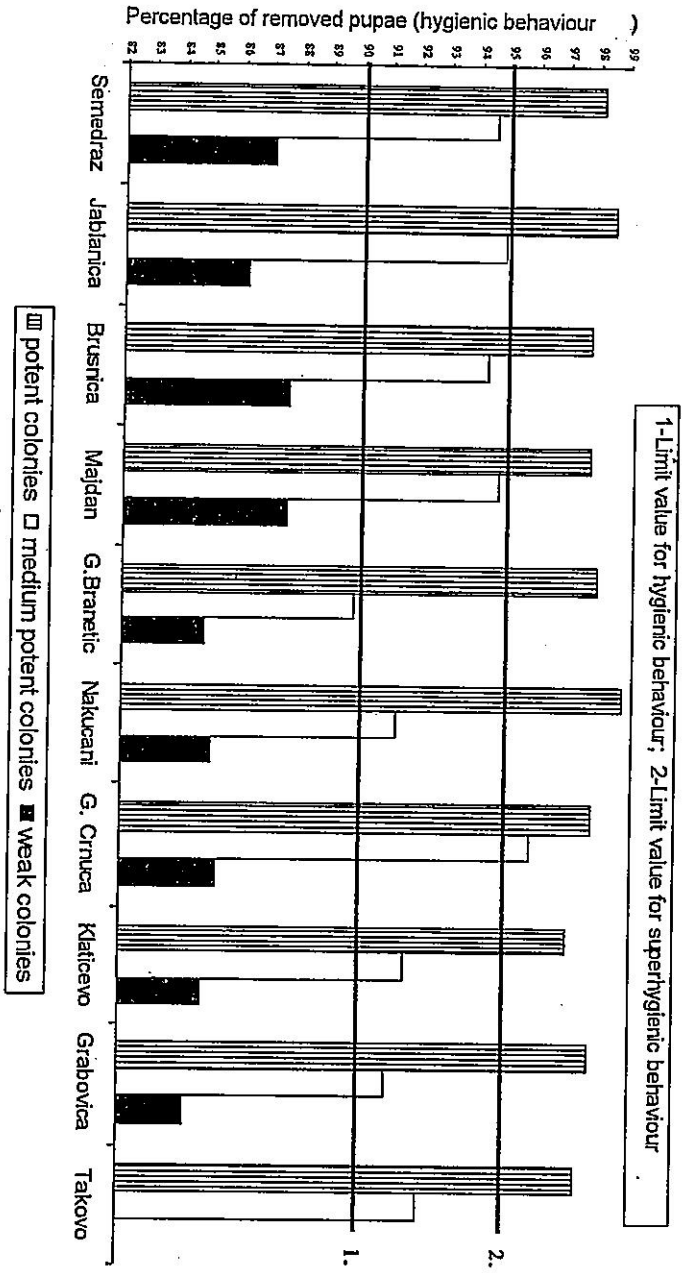

Figure 4. Comparison of honeybee hygienic behaviour at 10 different localities in the Rudnik region

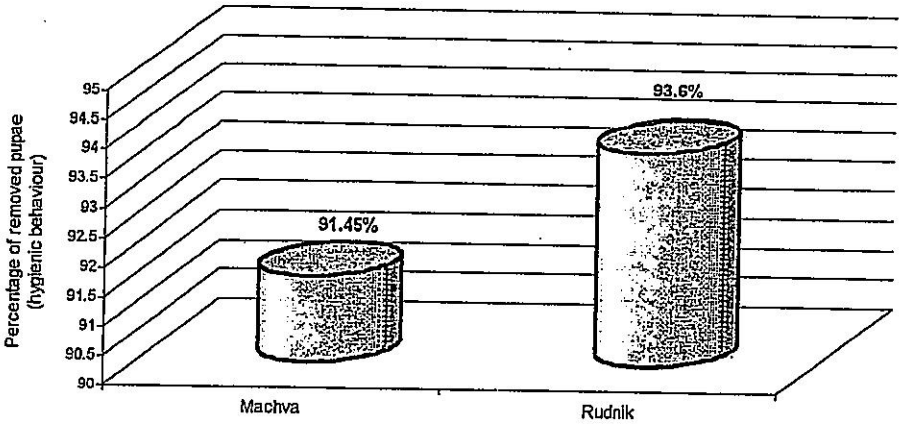

Figure 5. Comparison of hygienic behaviour between honeybee colonies from Machva and Rudnik regions

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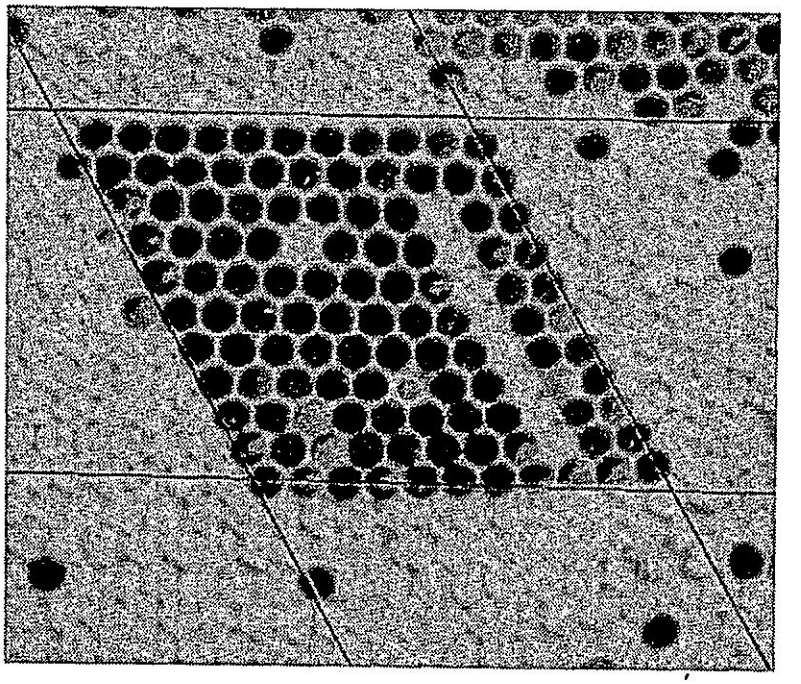

Figure 6. The example of a cleaned comb in a potent colony from Stitar locality, with expressed hygienic behaviour (91.73%).

Descriptive analysis was applied for identification of differences in the level of exertion of the hygienic behaviour between grey bees from Rudnik and yellow bees from Machva. This enabled us to affirm differences in expression of the studied feature between colonies from the mentioned regions (Table 1). Meanwhile, for economic beekeeping it is not necessary to study differences in the level of exertion of the hygienic behaviour between medium potent and weak colonies, within medium potent colonies and within weak colonies. Hence, the LSD test was applied only to affirm the level of exertion of the hygienic behaviour between potent honeybee colonies with one-year old queens and potent colonies with two-year old queens from both analysed regions, which included all individual inter- comparisons (Table 2). The LSD test revealed statistically highly significant differences ($p<0.01$) in the exertion of hygienic behaviour between potent colonies with one-year old and two-year old queens from the Rudnik region and potent colonies with one-year old and two-year old queens from the Machva region in favour of grey bees from the potent colonies from Rudnik (Table 2). There was no difference in the level of exertion between potent colonies with one-year old and two-year old queens within the Rudnik region nor within the Machva region.

Table 1. Comparison of hygienic behaviour between potent honeybee colonies from Machva and Rudnik regions.

	X	SD	SE	Cv (%)	min - max
A_1	117.17	2.3444	0.2344	2.00	112 - 121
B_1	116.81	2.4441	0.2444	2.09	111 - 121
A_2	118.63	1.8730	0.1873	1.58	113 - 121
B_2	118.48	1.7378	0.1738	1.47	114 - 121

A_1 - potent colonies with one-year old queens from Machva region
B_1 - potent colonies with two-year old queens from Machva region
A_2 - potent colonies with one-year old queens from Rudnik region
B_2 - potent colonies with two-year old queens from Rudnik region

Table 2. Comparison of differences in hygienic behaviour between potent honeybee colonies from Machva and Rudnik regions.** ($p < 0.01$)

Compared colonies	X	A_1	B_1	A_2	B_2
B_2	118.48	-1.31**	-1.67**	0.15	
A_2	118.63	-1.46**	-1.82**		
B_1	116.81	0.36			
A_1	117.17				

A_1 - potent colonies with one-year old queens from Machva region
B_1 - potent colonies with two-year old queens from Machva region
A_2 - potent colonies with one-year old queens from Rudnik region
B_2 - potent colonies with two-year old queens from Rudnik region

DISCUSSION

The obtained results for hygienic behaviour revealed that the honeybee colonies from the Rudnik region have more active hygienic behaviour than similar colonies from the region of Machva (Figure 5). Analysis of variance showed the existence of group differences, whereas the LSD test detected the presence of statistically highly significant ($p<0.01$) differences in the monitored behaviour between the analysed honeybee colonies from the two regions, in favour of colonies originating from Rudnik localities. The overall results of the analysis of hygienic behaviour in honeybee colonies showed that yellow bees from the Machva region as well as grey bees from the Rudnik region belong to the category of hygienic colonies, as the efficiency of elimination of damaged pupae amounted to 91.45% in Machva honeybees, and 93.60 % in Rudnik honeybees (Figure 5). Our results point to an indisputable relationship between hygienic behaviour and the strength of honeybee colonies, i.e. the potent colonies have more expressed hygienic behaviour. These results are in agreement with those of Spivak & Downey (1998), Spivak & Reuter (1998) and Stanimirović *et al.* (2000, 2001a, 2001b, 2001c)

It can be concluded that the hygienic behaviour of the investigated yellow bees from Machva and grey bees from Rudnik, is a real and special potential, being more expressed at the population level, as supported by the observed high intra- and interpopulation variabilities of the studied feature. These conclusions are in agreement with those of Stanimirović *et al.* (2000, 2001a, 2001b) and Pejovic (2001).

The obtained data concerning hygienic behaviour of yellow and grey bees in Serbia, especially the finding that variability is higher at the population level (intra- and interpopulation variability) compared with global expression of this feature in bees from the investigated regions, indicate that the investigated behaviour is genetically unfixed and a polygenic feature. Therefore, the level of hygienic behaviour is not obligatorily inherited from parents to offspring. Our interpretation of the results confirms those of Kefuss *et al.* (1996), Spivak & Reuter (2001), and Stanimirović *et al.* (2000, 2001a, 2001b).

The superhygienic potent honeybee colonies (regardless of queen age) found at 9 investigated localities in the Machva region and at 10 investigated localities in the Rudnik region could be used as breeding colonies for rearing quality queens. These queens would produce progeny with highly expressed hygienic behaviour and thence a greater resistance to diseases. Spivak & Gilliam (2002) stated that investigations of hygienic behaviour are very important for conserving honeybee genetic diversity using supermated queens, because drones for instrumental insemination are chosen from colonies expressing desirable traits such as a high level of hygienic behaviour and disease resistance (superhygienic colonies). Supermated queens have very high brood viability due to the high diversity of sex alleles, which means greater diversity. By maintaining a high degree of genetic diversity the negative effects of inbreeding are avoided. Supermated queens contain many times the genetic diversity of naturally mated queens. They are excellent as breeder queens in breeding programs to prevent accidental inbreeding. After all, we can conclude that those superhygienic colonies represent a remarkable genetic resource for starting organic beekeeping in Serbia (Vučinić *et al.*, 2002).

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ISTRAŽIVANJA HIGIJENSKOG PONAŠANJA I OTPORNOSTI NA BOLESTI KOD DVA EKOGEOGRAFSKA VARIJETETA MEDONOSNIE PČELE

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SADRŽAJ

Higijensko ponašanje kranjske medonosne pčele (*Apis mellifera carnica* Pollm.) ispitivano je na 20 lokaliteta u Srbiji (10 sa područja Mačve i 10 sa područja Rudnika). Rezultati su pokazali da sive pčele sa područja Rudnika imaju više ispoljeno higijensko ponašanje u odnosu na žute pčele sa područja Mačve. Dobijeni podaci ukazuju da društva oba ispitivana varijeteta medonosne pčele, sa područja Mačve i Rudnika, pripadaju kategoriji takozvanih higijenskih kolonija, obzirom da je efikasnost eliminacije oštećenih lutki iznosila 91,45% kod pčela u Mačvi i 93,60% kod pčela sa Rudnika. Naši rezultati ukazuju i na nedvosmislenu povezanost higijenskog ponašanja i jačine pčelinje zajednice, što znači da jača društva imaju više izraženo higijensko ponašanje.

Oba ispitivana varijeteta medonosne pčele mogu se koristiti za osvežavanje visoko selekcionisanih linija, kao i za organsko pčelarenje u Srbiji, zahvaljujući ispoljenom higijenskom ponašanju što podrazumeva i rezistenciju na bolesti kao što su varoza, američka kuga i naročito krečno leglo.