Scientific Papers. Series D. Animal Science. Vol. LXII, No. 1, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PARTICULARITIES OF NATURAL *Varroa* - RESISTANCE OF HONEYBEES POPULATION OF CARPATHIAN RACE FROM THE CODRI OF THE REPUBLIC OF MOLDOVA

Valentina CEBOTARI[1], Ion BUZU[1], Olga POSTOLACHI[1], Adrian SICEANU[2], Eliza CĂUIA[2]

[1]Institute of Zoology. 1, Academiei str., MD-2028, Chisinau, Republic of Moldova
[2]Institute for Beekeeping Research and Development, Ficusului Avenue, No. 42, Sector 1, Bucharest, Romania

Corresponding author email: valentinaceb@yahoo.com

Abstract

The aim of the researches was to reveal the particularities of natural varroa-resistance of honey bees of Carpathian race from the Moldavian Codri and their use in the selection and genetic amelioration of the local bee subpopulation. Scientific researches were a part of the SMARTBEE / FP7.Eu-KBBE.2013.1.3-02 Sustainable Management of Resilient Bee Populations project, in collaboration with the Institute for Beekeeping Research and Development in Bucharest, Romania. In order to achieve the purpose, at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova an experiment was carried out, on a batch of 25 bee families, choosed randomly, supplemented with young queens, obtained conducting from the same apiary. The experiment lasted three years (March 2015-March 2018), in which the queens were not replaced, and the bee families during the entire experimental period were not subjected to any anti-varroa or other disease treatment, nor additional feeding in spring or autumn with nutritional stimulators has not been applied. It was found that under natural conditions, without anti-varroa drug interventions and without the change of queens, from 25 colonies at the begining of experiment only 11 survived, which is 44%. In the bee families that survived in the third year of experiment, the index of natural Varroa mite fall during September-October was higher, compared to the first-year bee families by 42.7-43.0% (t_d=2.3-1.3; P<0.05 and P>0.1) and 2.7-3.0 times compared to the bee families of the second year (t_d=4.9-3.0; P<0.001 and P<0.01). Infestation degree of bees with Varroa mite increased, reaching peak values in the third experimental year, up to 4.81 ± 1.00 mites/10 grams of bees, which led to the inhibition of the main physiological functions of reproduction and developing of bee families. The infestation of capped brood cells with Varroa mite progressed in the third year of experiment, being significantly higher in September by 11.5% (td=2.9; P<0.01), compared with the same month of the previous year, which lead to a decrease in the quantity of capped brood, of the colonies strength and the weakening of the vital activity of the bee families as a whole. Queens prolificity declined significantly in October from 700 eggs/24 hours in the first year, to 234 eggs/24 hours in the third year, being less in the last year with 466 eggs/24 hours, or 66.6% (td=5.1; P<0.001). Out of the 11 bee families, which survived in natural conditions for three years, 5 more valuble families were selected in the breeding batch for reproduction. The bee families of the breed batch significantly exeeded the families from experimental batch after the queens prolificity – with 18.4% (P<0.05), the amount of capped brood – with 18.6% (P<0.05) and honey production – with 6.8% (P<0.01). At the same time, in the bee families of the breeding batch, the degree of infestation with mites was lower, the index of natural mites fall and the colonies strength – higher, as well as the boosted wintering resistance, ranging from 82.3-89.0%.

Key words: *bees, natural varroa-resistance, longevity, honey.*

INTRODUCTION

Varroa destructor mite is part of one of the most invasive parasitic species that attacks the most valuable, from a productive-useful point of view, species of insects such as *Apis mellifera* L. The *Varroa* mite's female, parasiting on the body of drones and worker bees and feeding the hemolimph of these hosts, lay their eggs in the combs with uncapped brood cells, preferring the drone brood. The larvae of the mite, feeding intensely with the hemolimph of the larvae and nymphs of bees, reaches within a very short time (4-7 days) the adult form.

Their mating occur in the brood cell. With the bee hatching, the *Varroa* female migrates throughout the hive, and sitting on the body of the drones and bees, the cycle of meta-morphosis is continued again. Infested bees have a shorter life and their productivity is diminished. Brood with low infestation of mites gives rise to small bees with low viability. Heavy *Varroa* mite infestation leads to the appearance of unviable bees with undeveloped wings, deformed head and feet. They fall on the bottom of the hive and are removed outside by healthy bees.

The heavily infested drones lose their mating behavior and the queens remain unfertilized (Ogradă, 1986; Calderon et al., 2010; Guzman-Novoa et al., 2010; Neuman et al., 2010). In addition, by perforating the bee's cuticle, *Varroa* mite inoculates a series of viruses and fungi, triggering the development of a range of quite contagious diseases at bees (Savu, 2012; Dainat et al., 2012; Nazzi et al., 2016; Guzman-Novoa et al., 2012).

The damaging impact of this invasive parasite species on the apiculture branch, as a whole, is of enormous dimensions (Lee et al., 2015; Buchler et al., 2014).

Our previous researches (Cebotari et al., 2013) demonstrated that the intensity of this invasion (calculated by the number of mites per 100 drone brood cells) in *Apis mellifera* bees families averages from 20.7±2.1% in apiaries where attention is paid to prophylaxis and disease control, up to 28.0±1.5% in apiaries where less attention is paid to prophylaxis and disease control measures. The degree of infestation, calculated from the number of mites localized on the bee's body, per 100 worker bees, averaged from 8.9±0.9% to 14.8±0.4% on the studied apiaries. Heavy *Varroa* mite infestation has a negative impact on the vital activity and productivity of the bee family. The coefficient of regression of the amount of honey extracted from the nest, depending on the infestation degree of bee family by *Varroa* parasite is: $R_{x/y}=1.60\pm0.07$ ($t_r=24.2$; $P<0.001$). This means that as the *Varroa* infestation rate increases by 1%, the amount of honey extracted from the nest is reduced by 1.6 kg. For these reasons, beekeepers, specialists and scientists in the field always are looking for effective methods to control and combat this disease in the honey bee (DeGrandi-Hofman et al., 2017; Wantuch et al., 2009).

Some specialists (Oddie et al., 2017; Buchler et al., 2014) drew attention to the ability of bees to respond to the invasive attack of the *Varroa destructor* mite, thereby highlighting their natural resistance against the aggressive parasite.

The natural varroa-resistance of melliferous bees is a phenotypic trait (variability), as in any animal species, resulting from the interaction of genetic variability of the animal population with environmental conditions (Runderer, cited by Siceanu, 2012). At any apiary, under similar conditions of maintenance, exploitation and environment, a variability of bee families is observed after a string of morpho-productive characters, such as: wintering resistance, queen prolificity, amount of capped brood, colony strength, and, finally, the production of honey. The manifestation of these morpho-productive characters in bee families is dependent on their ability to resist against pathogens of different origins: virotic, bacterial, fungal, parasitic, etc. Even under the conditions of systematic drug treatments, some bee families are more vulnerable, others more vigorous against actions of pathogenic factors. These skills are genetically inherited naturally.

According to some researchers in the field (Rosenkranz et al., 2010; Buchler et al., 2014), in European honey bees races, about 5-20% of mites, which infest a bee family, remain infertile after entering in the brood cells. Moreover, there is delayed laying of eggs in some mites, correlated with the development of bee brood, or lay only eggs from which only males come out because they are not fertilized. These situations limit the reproductive success of *Varroa*, which can be measured as the number of female daughters resulting from an adult female (founder) at the time of the brood emerging. Some researchers (Harbo et al., 2015; Panziera et al., 2017) have identified in some bee families a relatively high percentage of non-reproductive mites, which is a heritable trait of the honey bee and which they called "*Suppression of Mite Reproduction - SMR*". It was found that the low percentage of fertile mites was due to the bee's preferential removing of reproductive mites, therefore, this character was renamed "*Varroa sensitive hygiene - VSH*". The investigations of these authors have completed the idea that SMR can also come from other mechanisms, such as the ability of the brood to limit reproduction of the mites. So, some of the bee families may have high levels of mite resistance. They are of interest in the selection of colonies with natural varroa-resistance.

According to Locke et al. (2012), in some populations selected by *Apis mellifera* in Europe, non-reproductive mites were found in a significant proportion (40-50%), a character appropriated by heredity. Therefore, it is about varroa-resistance of bees.

At the same time, existing researches in this field does not provide sufficient information on the conditions in which bee populations with high varroa-resistance were selected (with or without drug treatments, duration of selection period, longevity of bee colonies in natural conditions without changing queens, evolution of the main morpho-productive characters, etc.) as well as how this high resistance to the productivity of bee families is reflected. In this context, the aim of our researches was to reveal the particularities of natural varroa-resistance of honey bees of Carpathian race from the Moldavian Codri and their use in the selection and genetic amelioration of the local bee subpopulation.

MATERIALS AND METHODS

The scientific researches were carried out on bee families *Apis mellifera carpatica,* at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova, located in the central part of Moldovan Codri, Forest District Ghidighici, Canton no. 8, Forest Sector no. 21. The main honey plants sources in the area were white acacia (*Robinia pseudoacacia*), large-leaved linden (*Tilia platyphyllos*) and polyphlora of wild plants growing around the forest massifs.

For investigation, in the spring of 2015, an experimental batch of 25 similar bee families was formed, in which young queens were introduced. The bee families of this batch were grown and exploited until spring (March) 2018 without anti-varroa drug treatments and without changing the queens. In the experiment the evolution of natural varroa-resistance of bee colonies without human drug intervention was monitored. During the experimental period (March 2015 - March 2018), a series of indicators and morpho-productive characters for each bee family, wich characterize the natural varroa-resistance and productive performance were evaluated, such as: index of the natural fall of the *Varroa* parasite, the degree of infestation of bees with parasites, the degree of infestation of the brood with *Varroa,* queen prolificity, family strength, the amount of capped brood, wintering resistance of bee colony, the survival of bee families throughout the experiment and the production of honey accumulated in the nest.

The natural fall index of the *Varroa* parasite was assessed on the sticky board with greased paper, located in each hive for a 48 hours, after that the mites were counted.

To determine the degree of infestation of bees with the *Varroa* parasite, the Icing Sugar method was applied. For this, from the honey combs 40-50g of bees were taken of each bee family. The bees were placed in plastic recipients about 750 ml covered with a rare sieve cover through which they did not pass, being weighed with the container. Then, in a 150 ml plastic beaker, 5 tablespoons of powdered sugar were added and poured over the bee pot nets, shaking lightly so that the sugar bears the bees well. For 3 minutes the bee and sugar recipient was shaken from time to time. After this treatment, all mites detach the bees and fall into powder. Subsequently, the recipient was inverted and shaken on a 2.8 mm mesh sieve. After sifting, powdered a czut, and the mites remained on the sieve, being easily counted on a white foil. The bees left in the container were shaken in the family from which they were extracted. The degree of infestation of bees with the *Varroa* parasite was calculated by multiplying the number of mites, found in the jar to 10, and the obtained result was reported to the net bee mass in the recipient.

To determine the degree of infestation of the brood in the nest, a comb with capped brood in the young puppy stage (white or pink eyes) was selected, and about 50 cells were uncapped linearly, in which was then verified the presence or absence of *Varroa* mites. The number of cells infested with *Varroa* in relation to the number of investigated cells is the brood infestation degree, expressed as a percentage.

The queen prolificity was determined according to our methods (Cebotari et al., 2010) described in the Zootechnical norme regarding evaluation of bee families, the

growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011.
The amount of capped brood was determined with Netz frame (5x5 cm) of the surface occupied by capped brood, expressed in the number of Langstroth frames with the precision of tenths of the frame.
Strength of the bee family was determined by appreciating the number of compact occupied by bees intervals in the nest, expressed in tenths of frames. The wintering resistance of the bee families was assessed by the amount of surviving bees during wintering, using the data from the autumn revisions of the previous year and the spring of curent year. The ratio of the amount of bees after wintering to the amount of bees at the begining of the winter, expressed as a percentage, represents wintering resistance.
Survival of bee families has been evaluated in the spring of each year during the entire experiment period. The ratio of survived bee family in the spring of this year to the number of bee families existing at the start of the experiment, expressed as a percentage, shows the survival index of bee families.
Honey production was evaluated by the method of examining the amount of capped honey accumulated in the nest after each basic harvest and the conversion of the number of honey frames into kilograms.
Selection of the most valuable bee families in the breeding group was performed taking into account longevity, stability of queen prolificity, varroa resistance, winter resistance, colony strength and honey production.
The data obtained as a result of calculating the average value of morpho-productive characters and comparing the differences of two variables were statistically processed using the computerized software "STATISTICA-12" and evaluated their certainty, according to variation biometric statistics, by methods of Плохинский (1989).

RESULTS AND DISCUSSIONS

The results of the research have shown that bee families, studied for three years of growth, without anti-varroa medication, showed a natural varroa resistance specific to the population of the ecotype formed in this area. From the beginning it should be mentioned that under these conditions, after the first year of experiment, 23 families out of the 25 have survived, which represents 92% (Table 1).

Table 1. Indices of natural *Varroa* resistance and bee family development characters at different times of the year

Investigated characters	08 August	29 August	19 September	10 Octomber	29 Octomber	20 March of next year
Year 2015 (N=25)						(N=23)
Natural fall of mites in 48 hours, mites / bee family	15.6±1.4	15.9±1.3	15.9±1.2	15.1±1.3	14.1±1.3	5.3±0.3
Degree of infestation of bees, mites / 10 g bees	3.03±0.28	3.28±0.29	3.00±0.26	2.81±0.24	2.94±0.33	0.96±0.07
Degree of infestation of brood, % of infested cells	29.9±2.4	34.4±2.8	38.2±3.1	37.3±4.3	-	8.0±0.5
Queen prolificity, eggs / 24 h	2370±60	2500±59	2080±55	700±78	-	1598±24
The amount of capped brood, no. frames with the brood	4.74±0.12	5.00±0.12	4.16±0.11	1.40±0.16	-	3.20±0.05
Colony strength, no. frames with bees	14.8±0.2	11.8±0.2	8.1±0.1	6.7±0.2	6.1±0.2	5.0±0.1
Year 2016 (N=23)						(N=16)
Natural fall of mites in 48 hours, mites / bee family	5.2±0.6	7.7±0.6	8.5±0.9	7.2±0.9	7.1±0.8	2.6±0.7
Degree of infestation of bees, mites / 10 g bees	1.60±0.23	1.06±0.19	1.09±0.20	0.74±0.17	0.80±0.14	0.97±0.16
Degree of infestation of brood, % of infested cells	4.1±0.7	5.4±0.5	5.7±0.9	4.5±0.9	5.3±1.2	3.4±1.2
Queen prolificity, eggs / 24 h	1719±31	2091±44	1391±37	650±53	119±32	909±65
The amount of capped brood, no. frames with the brood	3.44±0.06	4.18±0.09	2.78±0.07	1.30±0.11	0.24±0.06	1.82±0.13
Colony strength, no. frames with bees	13.6±0.2	9.5±0.2	7.8±0.2	6.4±0.2	5.7±0.3	5.1±0.2

Year 2017 (N=16)						(N=11)
Natural fall of mites in 48 hours, mites / bee family	3.1±0.7	10.8±1.8	22.7±2.7	21.6±4.8	14.5±5.0	5.1±2.1
Degree of infestation of bees, mites / 10 g bees	1.55±0.14	2.36±0.24	4.54±0.69	4.81±1.00	4.03±1.03	1.04±0.21
Degree of infestation of brood, % of infested cells	4.1±1.6	11.4±3.3	17.2±3.9	9.0±2.7	4.7±1.5	4.9±1.1
Queen prolificity, eggs / 24 h	1672±67	1969±99	1953±103	234±48	89±0	1355±76
The amount of capped brood, no. frames with the brood	3.34±0.13	3.94±0.20	3.91±0.21	0.47±0.10	0.18±0.0	2.71±0.15
Colony strength, no. frames with bees	8.8±0.1	7.9±0.3	7.0±0.3	5.9±0.4	5.8±0.4	5.6±0.2

After the second year of experiment, 16 families of bees survived, representing 64% of the 25. After the third year of the experiment, only 11 bee families survived, which is 44%. These colonies were most resistant in the to natural growth conditions, without anti-varroa antropic intervention. We found out that in the first year of the experiment the bee families removed *Varroa* mites quite actively, which could be noticed on the sticky board, especially installed in the hive. Thus, the index of natural fall of mites varied in the first year from 14.1 to 15.9 mites in 24 hours/bee family. The number of naturally fallen mites in the second year of experiment decreased by about two times, being from 5.2 to 8.5 mites in 24 hours/bee family, and then again increased in the third year of experiment, reaching the level of the first year and being in different periods of the year from 3.1 to 22.7 mites in 24 hours/bee family.

It was found that over the three years of experiment, the index of natural *Varroa* mite fall had the highest values each year in September, constituting 15.9±1.2 in 2015, 8.5±0.9 - 2016 and 22.7±2.7 mites in 24 hours/bee family in 2017. We should be mentioned that, the natural fall of mites in the second year of experiment was lower than in the first year with 7.4 mites or 46.5% (t_d=4.9; P<0.001). In all research years, since March, the index natural *Varroa* mites fall was increasing until the end of September, after which a decrease was observed. If, in 2015, the natural fall of mites increased insignificantly (only by 1.9%) during August-September, then in the following year (2016) it increased by 63.5% and in the the third year (2017) - by 7.3 times. After September, the index of natural Varroa mites fall decreases by the end of October by 11.3% - in the first year, by 16.5% - in the second year and by 36.1% in the third year of experiment. We found that in bee families that remained alive in the third year of experiment, the index natural mites fall during September-October is higher compared to first-year bee families by 6.8-6.5 mites, or 42.7-43.0% (t_d=2.3-1.3; P<0.05 and P>0.1) and, compared to the second year bee families, by 14.1-14.4 mites, or 2.7-3.0 times (t_d=4.9-3.0; P<0.001 and P<0.01).

On the basis of these data, we can conclude that at the bee families that remained alive in the third year of experiment, whithout anti-varroa drug treatment, the instinct of natural cleansing (discharging) by *Varroa* mites is quickens, compared with the first bee families and two experiment. Or, after three years of experimentation, we can say that the greatest chances of survival, given the lack of anti-varroa treatments, have those colonies where the cleasing behavior instinct against *Varroa* mites is more developed. We believe that they have a higher natural varroa-resistance.

Analyzing the degree of infestation of bees with *Varroa* mite we found that, every year after the wintering period, at the first spring revision (March), the degree of infestation of bees with mites is small and constitutes 0.96-1.04 mites/10 g bees. However, as the air temperature rises, the degree of infestation of bees with *Varroa* mite increases, reaching values ranging from 2.81-3.28 mites/10 g of bees in 2015 in August, 0.74-1.60 mites/10 g bees in 2016 and 1.55 to 4.81 mites/10 g bees in 2017. In the first two years of experiment, the degree of bee infestation decreased, from 3.03-3.28 mites/10 g bees in August to 2.81-2.94 mites/10 g bees in October of 2015 and from 1.60-1.06 mites/10 g bees in August to 0.74-0.80 mites/10 g bees in October 2016. In the third year of experiment (2017), the degree of infestation of bees with *Varroa* mite increased significantly from 1.55-2.36 mites/10 g of bees in August to 4.81-4.03 mites/10 g bees in October. This demonstrates that, despite the fact that bees possess the natural cleasing behavior against *Varroa* mite, the invasion of this aggressive parasite under natural conditions, without drug interventions, extends predominantly. In the autumn of the third experimental year, at the beginning of October, the infestation rate of bees with *Varroa* mite reached peak values, which averaged over the total remaining bee families (N=16) 4.81±1.00 mites/10 g bees. This leads to inhibition of the main physiological functions of reproduction and development of bee families.

Analyzing the degree of infestation with the *Varroa* mite of capped brood cells, we found that during the year the evolution of this indicator is increasing from March (spring revision) to August-September, with a further decrease in the end of October. The experiment data showed that the highest infestation rate of the brood was established in the first year. Of the total brood cells investigated in the first year, they were infected with the parasite *Varroa* 34.4% at the end of August, 38.2% in September and 37.3% at the beginning of October. In the second year of experimentation, the degree of infestation with the *Varroa* mite of brood cells, in the remaining in the experiment bee families (N=23), decreased to 4.1-5.7%. In the third year of the experiment, in the remained alive bee families (N=16), this indicator rose again to 4.7-17.2%. During 3 years of research it was established that the infestation of brood cells with *Varroa* mite reached the highest values in September. Thus, the *Varroa* infestation degree of brood cells in September 2015 was higher by 8.3% compared to the beginning of August (t_d=2.1; P<0.05). In 2016, infestation with the Varroa mite of cell brood in September had a tendency to be higher than that at the beginning of August. In 2017, the infestation degree with *Varroa* mite of brood cell in September was significantly higher than at the beginning of August, with 13.1% (t_d=3.1; P<0.01). Based on the obtained experimental data, we can say that despite the fact that the honey bees possess the instinct of the hygiene preferentially remove of reproducrive mites, also called *Varroa sensitive hygiene* - VSH, however, without the anti-varroa treatments, infestation of the brood progresses threateningly in the third year of experiment. Thus, the degree of infestation with *Varroa* of brood cells in August 2017 was higher with 6.0% (t_d=1.8; P<0.1) compared with August 2016, and that of September 2017 was significantly higher than in the same month of 2016, by 11.5% (t_d=2.9; P<0.01).

Increasing the *Varroa* mite infestation of bees and brood lead, finnaly, to the weakening of the vital activity of bee families, expressed by diminishing of the prolificity of the queens, the amount of capped brood and the straigth of the colonies.

Thus, the queens prolificity during three years of experiment decreased significantly in August from 2370 eggs/24 hours in the first year, to 1672 eggs/24 hours in the third year of experiment, being lower by 698 eggs/24 hours, or 29.5% (t_d=7.7; P<0.001). In September, the prolificity of queens in the third year of experiment continued to decline. In October, the queen prolificity decreased considerably from 700 eggs/24 hours in the first year of experiment, to 234 eggs/24 hours in the third year of that period, being less in the last year with 466 eggs/24 hours, or 66.6% (t_d=5.1; P<0.001).

Over the three years of experiment, the amount of capped brood, grown in the nest of the colonies in August, decreased from 4.74 frames with brood in the first year up to 3.34 frames in the last year of experiment. The decrease of this morphological character was in this period on average with 1.4 frames with brood, or 29.5% (td = 8.2; P <0,001). In September, the amount of capped brood in the third year of experiment continued to decline. In October, the quantity of capped brood in the family nest decreased from 1.40 frames in the first year of experiment to 0.47 frames in the third year of this period, being lower in the last year by 0.93 frames, or 66.5% (t_d=4.9; P<0.001).

As a result of the decrease of the queens prolificity and the quantity of capped brood, the number of bees (family strength) hatched in the nest has also suffered. Thus, bee family strength over the entire experiment period declined significantly in August, from 14.8 bee frames in the first year of the experiment to 8.8 frames in the third year of experiment, the decrease being 8.6 frames, or 40.5% (t_d=39.3;

P<0.001). In September, the strength of bee colonies has fallen from 8.1 bee frames in the first year of experiment to 7.0 bee frames in the third year, declining by 1.1 bee frames, or 13.6% (t_d=3.4; P<0.01). In October, the decrease in bee family strength continued throughout the experiment period, constituting 0.8 bee frames, or 12.0% (t_d=1.8; P<0.1).

Same time, despite the stress conditions, in which bee families have been subjected during three years of experimentation, without anti-varroa drug treatments and without changing of queens, however, some of the colonies (11 families) survived until the spring 2018. We consider that these bee families have a natural resistance that allowed them to survive in extreme conditions. From this livestock, 5 breeding bee families were selected in the breed batch, with the numbers: 1, 7, 8, 33 and 49, intended for the collecting of genetic material and directed growth of the queens. We consider that these bee families have the most valuable indices of morpho-productive characters and natural varroa resistance, compared to the other families in the experimental batch (Table 2).

Table 2. The value of the morpho-productive characters and *Varroa* resistance of bee families selected in the breed batch, compared to the experimental group

Investigated characters	August 5015 (N=25)		September 2017 (N=16)		March 2018 (N=11)	
	Experimental batch N=20	Breed batch N=5	Experimental batch N=11	Breed batch N=5	Experimental batch N=6	Breed batch N=5
Natural fall of mites in 48 hours, mites / bee family	15.6±1.4	13.8±3.4	25.5±3.2	16.4±4.2*	5.0±3.0	5.2±3.2
Degree of infestation of bees, mites / 10 g bees	3.03±0.28	2.38±0.69	5.30±0.80	2.88±1.06*	1.11±0.27	0.94±0.35
Degree of infestation of brood, % of infested cells	29.9±2.4	25.6±4.2	20.5±5.1	10.0±4.7	4.7±1.8	5.2±1.4
Queen prolificity, eggs / 24 h	2370±60	2600±169	1863±123	2150±169	1292±119	1530±43*
The amount of capped brood, no. frameswith the brood	4.74±0.12	5.20±0.34	3.73±0.24	4.30±0.34	2.58±0.24	3.06±0.09*
Colony strength, no. frames with bees	14.8±0.2	15.4±0.5	6.7±0.4	7.4±0.3	5.5±0.4	5.8±0.2
Wintering resistance, %	80.0±1.6	78.8±3.6	79.8±0.9	82.3±1.2*	89.0±1.1	89.0±1.6
Survival of bee families, %	100	100	64.0	100	44.0	100
Honey production, kg	44.7±0.9	46.3±2.2	35.3±0.7	37.7±0.6**	-	-

Remarc: * - P<0,05 compared to the experimental batch.

At the basis of the principles of selection of bee families for breeding batch, the criteria of the development of their main morpho-productive characters have been set, such as: survival under natural maintenance conditions without antivarroa drug treatments and without changing the queen, stability of the queens prolificity over several years, the amount of capped brood in the nest, the colonies strength (the amount of bee present in the nest), the wintering resistance and the production of honey accumulated in the nest at the base harvest.

From the presented data it can be seen, at the end of the experiment, which coincided with the spring revision (March 2018), the bee families of the breed batch (N=5) significantly exeeded the families from experimental batch (N=6) after the queens prolificity - 238 eggs/24 hours, or 18.4% (t_d=1.9; P<0.05) and the amount of capped brood - by 0.48 frames with brood, or 18.6% (t_d=1.9; P<0.05). Same time, in the bee families of the breeding batch, the degree of infestation with mites was lower, but the index of natural mites fall and the colonies strength - higher. These bee families had a significantly higher honey production in 2017, compared to the families in the experimental group (N=11), with 2.4 frames, or 6.8% (t_d=2.6; P<0.01), as well as increased wintering resistance, ranging from 82.3-89.0%.

Analyzing the evolution of the morpho-productive characters of bee families in the breed group, compared to the families in the experimental group, during the three years of experiment, we found that the first had tendencies to overcome the level of character manifestation throughout the period. In our opinion, this is due to the fact that the bee families selected in the breed batch have a strong natural resistance to *Varroa*. This allows us to predict that from the genetic material of these colonies will get a descendents with boosted potential of natural varroa-resistance and high productivity skills.

CONCLUSIONS

The natural varroa-resistance of honeybees of the Moldovan Codri population allows survival of 44% of the bee families, maintained for three years in natural conditions, without anthropic anti-varroa drug treatments and without changing the queens.

During the experiment (3 years), it was established that the index of natural fall of *Varroa* mites in bee families is increasing from spring until the end of September, after which the decrease is occur until the end of October.

The index of natural mitesfall in bee families was higher in the first year of the experiment (14.1-15.9 mites per 24 hours/bee family), decreasing in the second year of experiment (up to 5.2 -8.5 mites in 24 hours/bee family), then increased significantly in the third year of experiment, reaching a maximum of 22.7 mites in 24 hours/bee family. In the bee families that survived in the third year of experiment, the index of natural *Varroa* mite fall during September-October was higher, compared to the first-year bee families by 42.7-43.0% (t_d=2.3-1.3; $P<0.05$ and $P>0.1$) and 2.7-3.0 times compared to the bee families of the second year (t_d=4.9-3.0; $P<0.001$ and $P<0.01$).

After three years of experiment, in which no anti-varroa drug treatments and no change of queens in bee families were applied, can survive only colonies with developed cleasing behavior instinct against *Varroa* mites.

Despite the fact that bees possess cleasing instinct against *Varroa* mite, however, under natural conditions without drug intervention, the degree of infestation of bees with mites increases, reaching peak values in the third experimental year up to 4.81±1.00 mites/10 g of bees, which leads to the inhibition of the main physiological functions of reproduction and development of bee families.

In the absence of anti-varroa medication, infestation of capped brood cells with *Varroa* mite progresses menacing in the third year of experiment, being significantly higher in September by 11.5% (t_d=2.9; $P<0.01$), compared with the same month of the previous year, which leads to a decrease in the quantity of capped brood, of the colonies strength and the weakening of the vital activity of the bee families as a whole.

Maintenance of bee families over three years under natural conditions without anti-varroa treatments and without change of queens leads to a significant decrease in prolificity of queens in October from 700 eggs/24 hours in the first year to 234 eggs/24 hours in the third year, being less in the last year with 466 eggs/24 hours, or 66.6% (t_d=5.1; $P<0.001$).

Out of the 11 bee families, which survived in natural conditions for three years, without anti-varroa drug treatments and without changing the queens, 5 families were selected in the breeding batch for reproduction.

The bee families of the breed batch significantly exeeded the families from experimental batch after the queens prolificity – with 18.4% ($P<0.05$), the amount of capped brood – with 18.6% ($P<0.05$) and honey production – with 6,8% ($P<0,01$). At the same time, in the bee families of the breeding batch, the degree of infestation with mites was lower, the index of natural mites fall and the colonies strength – higher, as well as the boosted wintering resistance, ranging from 82.3-89.0%.

ACKNOWLEDGEMENTS

Scientific researches were a part of the SMARTBEE / FP7.Eu-KBBE.2013.1.3-02 Sustainable Management of Resilient Bee Populations international project, with EU financial support, through the Institute for Beekeeping Research and Development in Bucharest, Romania, as well as within the fundamental institutional project 15.817.02.12F "Diversity, structure and functioning of complex natural and anthropogenic fauna in the context of strengthening of the national security strategy of the Republic of Moldova" funded from the state budget.

REFERENCES

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M.D., Conte, Y.L., Uzunov, A., Berg, S., Bienkowska, M., Bouga, M., et al. (2014). The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera L*. colonies in Europe. *J. Apic. Res*., 53, 205–214.

Calderon, R.A., Van Veen, J.W., Sommeirjer, M.J., Sanchez, L.A. (2010). Reproductive biology of *Varroa destructor* in Africanized honey bees (*Apis mellifera*). *Exp. Appl. Acarol*., 50(4), 281-297.

Cebotari, Valentina, Toderaş, I., Buzu, I., Postolachi, Olga (2013). Invasion of the *Varroa jacobsoni* parasite in *Apis mellifera* Carpatica bee families. Invazia parazitului *Varroa jacobsoni* în familiile de albine *Apis mellifera* Carpatica. *Buletinul Academiei de Științe a Moldovei, Științele vieții*, Chișinău, 3(321), 4-13, ISSN 1857-064X.

Cebotari, Valentina, Buzu, I. (2010). Zootechnical norms regarding the honeybee colonies evaluation, breeding and certification of genetic material in beekeeping. Contemporary Science Association. *Proceedings of the 1st International Animal Health Science Conference: The Beekeeping Conference*, Addleton Academic Publishers, New York, (Bucureşti), Library of Congress Control Number, 26-30.

Dainat, B., Evans, J.D., Chen, Y.P., Gauthier, L., Neumann, P. (2012). Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honey bees. *Appl. Environ. Microbiol*., 78: 981–987.

DeGrandi-Hoffman, G., Ahumada, F., Grahman, H. (2017). Are dispersal mechanisms changing the host-parasite relationship and increasing the virulence of *Varroa destructor* (*Mesostigmata Varroidae*) in managed honey bees (*Hymenoptera Apidae*) colonies. *J. Environ. Entomol*., 46(4), 737-746.

Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montano, L.G., Petukhova, T. (2012). Genotypic variabiality and relationships between mite infestation levels, mite damage, groming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L). *J. Invertebr. Pathol.*, 110(3), 314-320.

Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P.G., Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41, 443–450.

Harbo, J.R., Harris, J.W. (2015). Responses to Varroa by honey bees with different levels of Varroa Sensitive Hygiene Respuestas a Varroa de abejas melíferas con diferentes niveles de higiene sensible a la Varroa. *J. Apic. Res.*, 48, 156-161.

Lee, K.V., Steinhauer, N., Rennich, K., Wilson, M.E., Tarpy, D.R., Caron, D.M., Rose, R., Delaplane, K.S., Baylis, K., Lengerich, E.J., et al. (2015). A national survey of managed honey bee 2013–2014 annual colony losses in the USA. *Apidologie*, 46, 292–305.

Locke, B., Le Conte, Y., Crauser, D., Fries, I. (2012). Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations. *Ecol. Evol*., 2(6), 1144-1150.

Nazzi, F., Le Conte, I. (2016). Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. *Annu. Rev. Entomol*., 61, 417-432.

Neumann, P., Carreck, N.L. (2010). Honey bee colony losses. *J. Apic. Res*., 49, 1–6.

Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului Republicii Moldova. *Animal Breeding Standards regarding bee family bonuses, breeding and certification of beekeeping material*, approved by Government Decision of the Republic of Moldova, 306/ 28.04.2011 (M.O. al R.M. nr. 78-81 din 13.05.2011, art. 366).

Oddie, M.A.Y., Dahle, B., Neumann, P. (2017). Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection. *Peer J.*, 32, 262-271.

Ogradă, I. (1986). Bolile şi dăunătorii albinelor. *Bee diseases and pests*. Bucharest, RO: ACA-APIMONDIA Publishing House, 145.

Panziera, D., Van Langevelde, F., Blacquière, T. (2017). Varroa sensitive hygiene contributes to naturally selected varroa resistance in honey bees. *J. Apic. Res*., 56, 635–642.

Rosenkranz, P., Aumeier, P., Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *J. Invertebr. Pathol*., Jan. 103, Suppl. 1, S96-119.

Savu, V. (2012). Patologia albinelor. Apicultura – manualul cursantului. *Beekeeping - student's manual*. Ploieşti, RO: LVS Crepuscul, 176-214.

Siceanu, A. (2012). Ameliorarea și înmulțirea albinelor. *Apicultura – manualul cursantului*. *Beekeeping - student's manual*. Ploieşti, RO: LVS Crepuscul, 148-175.

Wantuch, H.A., Tarpy, D.R. (2009). Removal of drone brood from *Apis mellifera* (*Hymenoptera Apidae*) colonies to control *Varroa destructor* (*Acari Varroidae*) and retain adult drones. *J. Econ. Entomol.*, 102(6), 2033-2040.

Плохинский, Н.А. (1989). *Руководство по биометрии для зоотехников*. Изд. *Колос*, Москва, 256 с.