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## SPRING BROODING OF QUEEN BEES IN FAMILIES WITH VARYING INFESTATION WITH THE VARROA *DESTRUCTOR* PARASITE DURING AUTUMN PERIOD

**Summary.** Varroatosis is a contagious parasitic disease of the brood and all castes of mature bees caused by *Varroa destructor* mites. The presence of the parasite exerts a very negative impact on the bee colony. The objective of the investigations was to assess the effect of the degree of infestation of bee families during the autumn period on spring queen brooding evaluated by the measurement of brood area in these families. The total of 15 honey bee (*Apis mellifera*) colonies of *Carnica* breed was selected for investigations. They were divided into three groups of varying degrees of family infestation on the basis of the number of individuals of *V. destructor* which fell onto the bottom floor following the third fumigation of families with Apiwarol. In spring of the following year, four pictures of frames with capped brood were taken at three-week intervals using a digital camera. Next, using a computer digital image analysis program Microscan-Lucia, the outline of cells with sealed brood visible on the computer screen was made automatically allowing the determination of the sealed brood area. The obtained results were subjected to statistical analysis using for this purpose single-factorial analysis of variance, whereas the Student t-test was applied to compare differences. The performed calculations revealed absence of significant differences in the brood area in bee families affected to a different degree by varroatosis at different dates of measurements which indicated that the extent of infestation of bee families with the *V. destructor* parasite during the autumn period failed to affect the spring brooding of bee queens.

**Key words:** *Varroa destructor*, queen spring brooding

### Introduction

Varroatosis is a contagious parasitic disease of the brood and all castes of mature bees caused by *Varroa destructor* mites (ANDERSON and TRUMAN 2000). The first reports about the parasitosis of *V. destructor* on the honey bee date back to 1959 and

come from China (ROMANIUK 1998) and in Poland first cases were reported in 1980. At the present time, no continent is parasitosis-free and for many years now bee-keepers have been battling with this disease which is no longer restricted to individual countries but constitutes a global problem (FAKKIMZADEH 2001, GOODWIN and EATON 2001, BAGGIO et al. 2004). Varroatosis was placed by the International Office of Epizootics (frans. OIE) on the B list of animal contagious diseases. In Poland, on the strength of the Bill of April, the 24<sup>th</sup> 1997 dealing with the control of animal contagious diseases, examination of slaughter animals and meat as well as Veterinary Inspection, the above-mentioned disease is subject to compulsory registration (POHORECKA 2003).

The presence of the parasite has a very unfavourable influence on the bee family and visible symptoms of the disease include: dying of drone and bee brood, appearance of bees and drones with developmental disorders of wings, limbs and shortened abdomen. In addition, the disease is accompanied by the shortening of bee lives by 34-68%, dead or creeping bees in front of the bee-hive, intensification of the occurrence of other diseases such as: American foul brood, European foul brood invasion of wax-moth caterpillars and, last but not least, strong weakening of bee colonies at the end of summer and their massive deaths during the overwintering period, especially in the second and third years of the disease (IMDORF and CARRIERE 1996, ANDERSON 2000, AKYOL and KORMKAZ 2005, GLIŃSKI et al. 2007). Therefore, investigations on varroatosis have been carried out in various countries all over the world.

Following the performed extensive research work many different therapeutic agents as well as varroacidal preparations were developed. The effectiveness of the above-mentioned compounds were tested both in laboratory and field experiments (BIEŃKOWSKA 2007, CHUDA-MICKIEWICZ et al. 2007, AKYOL and YENINAR 2008). Unfortunately, with the passage of time *V. destructor* exhibits resistance to active ingredients, especially those which dissolve and accumulate in wax, such as, for example, pyrethroids (MILANI 1995, KANBAR and ENGELS 2003, KUBIK et al. 2000, VASELÝ 2003, KOPERNICKÝ and JENDREJÁK 2003). The resistance acquisition is possible already after several years of application of the same varroacidal preparations (LIPIŃSKI 2006).

The destruction of parasites found in a bee family is carried out at the present time not only with the assistance of various chemical preparations but also employing biological, physical and ecological methods. However, despite the application of integrated actions, there is no 100% preparation or method to control varroatosis and that is why investigations and experiments connected with the disease continue to be urgent and important for bee-keepers.

The aim of the study was to determine the impact of the degree of infestation of bee families with the *V. destructor* parasite during the autumn season on the spring brooding of queens in these families.

## Material and methods

Investigations were carried out in the autumn 2007 and spring 2008 on selected 15 families of honey bee (*Apis mellifera*) of Carnica strain which were settled in Wielkopolska type bee-hives. The selected families were characterised by similar strength (worker-bees settled six-seven frames). All queen-mothers in the experimental families

were two-year-old. All families were divided into three experimental groups depending on the degree of infestation with the *V. destructor* parasite.

During the first stage of investigations, autumn fumigations of bee families using Apiwarol preparation were applied. The preparation is manufactured by Biowet Company in Puławy and, at the moment, it is one of four certified agents to control varroosis available on the Polish market. The active ingredient in Apiwarol is amitraz and its quantity in one dose is 12.5 mg. The preparation was applied in the form of tablets for fumigation and the treatment was repeated three times at five-day intervals.

In the performed investigations, prior to the third fumigation, paper pads soaked in oil were placed on the floorboards of bee-hives which made it impossible for the fallen parasites to move. After the treatment, the number of fallen parasite females was counted and on the basis of the number of determined individuals, bee families were divided into three groups. Families in group I contained only three parasites, those classified as group II contained, on average, 45 individuals, while the average number of *V. destructor* females in group III was 430. Each of the three experimental groups comprised five bee families.

In spring 2008, measurements of sealed brood were carried out in order to determine the intensity of brooding of mother-queens. To ensure accurate determination of the examined area, pictures of honeycombs with capped brood were taken using a digital camera four times during the period from the 12<sup>th</sup> of March to the moment of appearance of first queen-cell cups in experimental families (until May 14<sup>th</sup>). Each picture was taken from identical distance so that the whole honeycomb could be seen on each photo. Pictures were taken every three weeks to avoid taking pictures of the same cells with sealed brood. Pictures of the sealed brood were taken on the following days: March the 12<sup>th</sup>, April the 2<sup>nd</sup>, April the 23<sup>rd</sup> and May the 14<sup>th</sup>.

In the course of the performed investigations, 547 pictures of sealed brood were taken. In the next step, the total area of sealed brood was measured using for this purpose a Microscan-Lucia software for computer image analysis. The program makes it possible to outline cells with sealed brood visible on the computer screen and allows automatic determination of the sealed brood area. Before measurements, the applied scale was calibrated on the basis of natural width of five cells on a honeycomb. Earlier methods of sealed brood area determination consisted in measuring it with the aid of a ruler directly on a honeycomb. That method was less precise and made it difficult to calculate the area accurately because both cells with capped and uncapped brood were present in the determined area of measurement.

In the performed statistical calculations, the authors applied single-factorial analysis of variance, while the Student t-test was used to compare the recorded differences.

## Results

The mean areas of the sealed brood in groups with different infestation by the examined parasite at individual dates of measurements are presented in Table 1. In order to show the results better, they were also presented in the form of a diagram (Fig. 1).

During the first measurement, the mean area of sealed brood in individual groups was at similar level and was contained in the interval from 230 and 293 cm<sup>2</sup>. This indicates

Table 1. Area of sealed brood in individual experimental groups at successive dates of measurements ( $\text{cm}^2$ )

Tabela 1. Powierzchnia czerwia krytego w poszczególnych grupach doświadczalnych w kolejnych terminach pomiarów ( $\text{cm}^2$ )

Date of measurement	Experimental groups		
	I	II	III
12.03	230	285	293
2.04	459	509	450
23.04	2 185	2 218	2 158
14.05	3 906	3 442	3 643

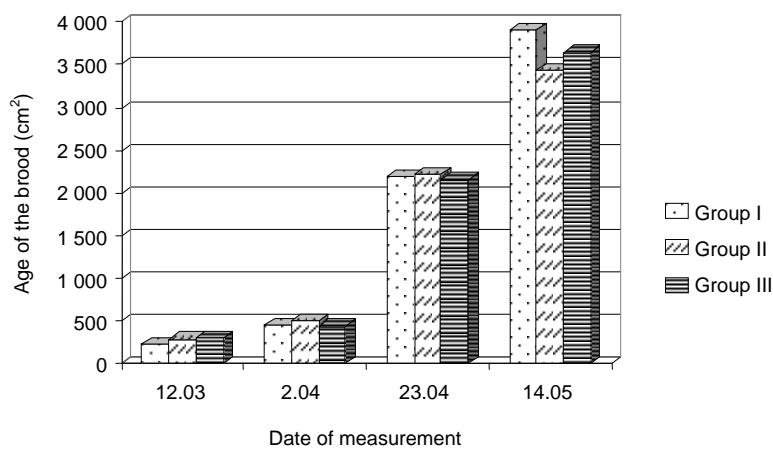


Fig. 1. Mean area of sealed brood at successive dates of measurements  
Rys. 1. Średnia powierzchnia czerwia krytego w kolejnych terminach pomiarów

that in March mother queens were brooding quite uniformly irrespective of what group they were in. On April the 4<sup>th</sup> and 24<sup>th</sup> bee families continued to develop on similar levels and brood measurements showed occupation of the area ranging from 450 to 509  $\text{cm}^2$  on the first April date, whereas on the second April date – the values fluctuated from 2158 to 2218  $\text{cm}^2$ . It was only on the last date of measurements that significant differences in mean sealed brood areas were observed in individual experimental groups. This area in group I reached 3906  $\text{cm}^2$  and it was the highest value and 3442  $\text{cm}^2$  in group II in which it was the lowest value. This indicates that the most intensive brooding occurred in families from the groups with the lowest infestation with *V. destructor*.

In order to establish whether varroatosis exerted a significant influence on the number of eggs laid by queen mothers, a single-factorial analysis of variance was carried out. The performed calculations revealed that no significant differences were found in

brood areas in families differently affected by varroatosis at different dates of measurements. The value of the F test amounted to 0.2 at the probability of 0.8 and it was lower than F tab.

On the basis of the performed sealed brood measurements it was also possible to estimate brooding intensity of queen mothers at individual dates as shown in Figure 2. It is evident that the strongest brood increment, i.e. increase of intensity of egg laying, took place between the 2<sup>nd</sup> and 23<sup>rd</sup> of April and it referred to all the examined experimental groups. During this period, the mean brood area increased nearly four times in comparison with the previous measurement date. Queen mothers continued to increase numbers of eggs laid by them but this increase was less intensive and did not exceed the interval 1.5 to 1.8 times in individual groups. This increase was highest in families characterised by the lowest autumn varroatosis infestation; however, the recorded differences were statistically non-significant as confirmed by the performed analysis of variance.

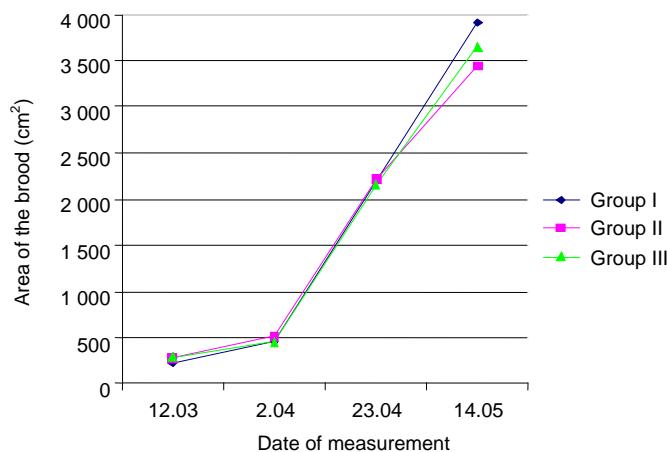


Fig. 2. Brooding intensity of bee queen mothers at successive dates of measurements

Rys. 2. Intensywność czerwienia matek pszczelich w kolejnych terminach pomiarów

## Discussion

Production potentials of bee families during the season are strongly influenced by the condition the family is in after winter. An exceptionally strong impact is exerted by the strength of the family in early spring (SKUBIDA and SKOWRONEK 1995) which can be checked on the basis of measurements of brood area. Earlier methods of sealed brood area determination consisted in measuring it with the aid of a ruler directly on a honeycomb (MARCINKOWSKI and SKUBIDA 1996). This method was less accurate in comparison with the one employed in this experiment and failed to calculate the surface accurately because both capped and uncapped brood cells were found in the determined field of measurements. In addition, brood increment dynamics, i.e. production potentials,

between consecutive measurements is also very important for bee families (SKUBIDA 1999, OLSZEWSKI 2007).

It is essential to identify properly the strength of the infestation with varroatosis because it is not very uncommon that as a result of incorrect assessment concerning the degree of infestation of bees with the parasite, less experienced bee-keepers delay the application of appropriate treatments unsuspicious that even 25 000 mature *V. destructor* mites can already live in apparently healthy bee families (FRIES 1994). This is particularly dangerous in view of the fact that already the presence of 6000 females of the discussed parasite may cause early death of an entire bee family usually with the arrival of autumn in the form of rapidly advancing steady loss of bees. Bearing in mind the fact that there may be over 1 million ( $25\ 000 \times 50 = 1\ 250\ 000$ ) *V. destructor* mites in apparently healthy families of a 50-hive apiary, it is not difficult to imagine what threat such an apiary poses for neighbouring bees, especially when we remember that during robberies, parasites from weaker, more infested families are transferred onto robbing bees.

It was also found that bees from more infected families migrate to less infected families. All this causes that properly treated and, hence, stronger bee families act as "lures" for *V. destructor* mites.

The fight against *V. destructor* mites, especially in areas densely populated with bee colonies, should be conducted in an organised manner (IMDORF et al. 2003). This type of procedure requires expert control so that all bee families in a given locality are treated within the framework of the same program. The basis of such programs is the prompt and punctual application of all curative treatments with preparations manufactured on the basis of synthetic contact acaricides which are characterised by appropriately high acaricidal activities in relation to the specific strain of *Varroa* which is to be destroyed (MARCANGELI and GARCIA 2004, LIPIŃSKI 2005). Reduced parasite populations, indirectly, lead to limited possibilities of the occurrence of viral, bacterial and fungal diseases which may develop in bees in connection with the pricking of larva and bee covers by feeding mite females (KANBAR and ENGELS 2003, 2005).

It is commonly known that inappropriate varroatosis control results in increased resistance of the parasite to drugs. For example, in Italy losses of families in regions of confirmed *Varroa* resistance reached 90% (BRATKOWSKI 2006). This was caused by massive application of highly effective synthetic contact acaricides.

Increased mite resistance and, consequently, increased numbers of parasites in a given family, despite the application of varroacidal preparations, leads to decreased honey production. Therefore, it is very important to control varroatosis effectively if high income from the apiary is expected.

As confirmed by the results of the described investigations, properly performed treatment of varroatosis control in the autumn period caused such a drop in the numbers of parasites that the remaining mites failed to have a significant impact on the spring brooding of queen mothers.

Losses of bee families observed in some regions of our country following an unexpected dynamics of proliferation of *V. destructor* mites should be taken as a warning to the remaining beekeepers. The problem of varroatosis control has not been solved yet and it should not be ignored; to the contrary, special caution and watchfulness should be maintained.

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## Conclusions

1. Different levels of infestation of bee colonies with the *Varroa destructor* parasite during autumn period, if appropriate effective measures of the parasite control are undertaken, do not affect spring brooding of queen mothers in these families.
2. The mean area of sealed brood in spring did not differ in the examined bee families irrespective of the degree of infestation of these families with varroatosis in autumn.
3. The intensity of egg laying by bee queens, i.e. the rate of family development, was highest in April irrespective of the autumnal degree of infestation of bee families with varroatosis.

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## WIOSENNE CZERWIENIE MATEK PSZCZELICH W RODZINACH O ZRÓŻNICOWANYM PORAŻENIU PRZEZ PASOŻYTA *VARROA DESTRUCTOR* W OKRESIE JESIENNYM

**Streszczenie.** Warroza jest zaraźliwą pasożytniczą chorobą czerwia i wszystkich kast pszczół dorosłych wywoływaną przez roztocza *Varroa destructor*. Obecność pasożytu ma bardzo niekorzystny wpływ na rodzinę pszczelą. Celem pracy było określenie wpływu stopnia porażenia rodzin pszczelich w okresie jesiennym na wiosenne czerwienie matek, ocenione pomiarem powierzchni czerwia w tych rodzinach. Do badań wybrano 15 rodzin pszczóły miodnej (*Apis mellifera*) rasy *Carnica*. Podzielono je na trzy grupy o zróżnicowanym stopniu porażenia na podstawie liczby osobników *V. destructor* opadłych na dennice po trzecim odymieniu Apiwarolem. Wiosną następnego roku wykonano czterokrotnie, w odstępach trzytygodniowych, zdjęcia ramek z czerwem krytym aparatem cyfrowym. Następnie za pomocą programu komputerowej analizy obrazu Microscan-Lucia dokonano obrysu komórek z czerwem krytym widocznymi na monitorze komputera, co pozwoliło automatycznie wyznaczyć powierzchnię czerwia. Uzyskane wyniki poddano analizie statystycznej (analiza wariancji jednoczynnikowej), a do porównania różnic użyto testu t Studenta. Obliczenia wykazały, iż brak jest istotnych różnic w powierzchni czerwia w rodzinach w różnym stopniu porażonych warrozą w poszczególnych terminach pomiaru, co oznacza, iż stopień porażenia rodzin pszczelich pasożytem *V. destructor* w okresie jesiennym nie ma wpływu na wiosenne czerwienie matek pszczelich.

**Słowa kluczowe:** *Varroa destructor*, wiosenne czerwienie matek pszczelich

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*Accepted for print – Zaakceptowano do druku:*

16.12.2009

*For citation – Do cytowania:*

Fliszkiewicz M., Walachowski M., 2010. Spring brooding of queen bees in families with varying infestation with the *Varroa destructor* parasite during autumn period. *Nauka Przyr. Technol.* 4, 1, #9.