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Comparison of components and number of *Nosema* sp. spores of wintering Carniolan and Italian bees debris

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Abstract: *Comparison of components and number of Nosema sp. spores of wintering Carniolan and Italian bees debris*. The aim of the experiment is to investigate and compare the components of the winter debris of two breeds of bees: Carniolan and Italian and microscopic tests of the bees samples from the experimental debris for the presence of *Nosema* sp. The study was carried on 20 wintering bee colonies in the apiary of the Apiculture Division of Warsaw University of Life Sciences – SGGW.

The research material was debris falling during the winter at the bottom of the hive that was bees, wax cappings, eggs, wax, pollen pellets, dark hardened fungal clods and fecal stains on the pads. The examination was conducted during the overwintering 2008/2009. In the spring of 2009 microscopic examination was conducted (samples of bees from the experimental debris for the presence of *Nosema* sp.). Pollen pellets are originated from bee bread, which bees use to feed the brood and was collected from two different breeds of bees. The presence of eggs in debris testifies to the fact that in winter queen of two studied breeds of bees are also lying eggs. The presence of fecal spots in debris is sporadic but it is the sign of nosemosis. Microscopic examination confirmed the assumption that the appearance of the fecal spots was a symptom of *Nosema* sp. presence. *Nosema* sp. infects bees of both breeds. Carniolan breed bees tend to self-medicate from nosemosis. Lack of feces does not indicate the lack of nosemosis in the case of both mentioned breeds of bees.

Key words: honeybee, winter debris, nosemosis, breeds of bees

INTRODUCTION

During winter bees do not leave the nest and accumulate feces in the rectum waiting for warmer days and for an opportunity to leave the hive and to empty bowels (Muszyńska and Bornus 1981). When the colonies in the autumn and winter time create a cluster, the activity of bees is reduced. They move at the small space within the comb and collect only a small amount of food needed to maintain the proper conduct of the necessary processes of life. Flights of bees are rare, but only during the transient (usually fleeting) warming. Sometimes at the end of February in our climatic conditions, also happen that temperature rises above 10°C, which bees use for the first flights for purification and water supply of hive (Muszyńska and Bornus 1981).

Bees do not clean their nests and do not throw away trash during winter. Falling to the bottom of the hives dead insects, food debris (pollen, honey, sugar), wax cappings, dried crumbs, pieces of propolis and other various organic molecules and inorganic contaminants (sand) accumulate in the form of debris (Chmielewski 1992). The practice

of wintering bees of different races in areas with harsh winters shows that the first debris is made up of bees that have fallen to the bottom and froze. The highest daily debris is observed during the first two months of overwintering hives, when there is the peak concentration of bees in the cluster and there is continuous replenishment of outer shell by the bees from a center of the comb. Debris in the second half of wintering consist of the bees died due to excessive physiological overload, with overfilled gut. Next appears the stability of thermoregulation violation in comb, increased food consumption, death of older bees in the colony, which is an important evaluation index of wintering colonies and their possible start to the new beekeeping season (Skubida 1994). According to Chmielewski (1991, 1992), on the basis of qualitative and quantitative debris composition can be conducted observations on the state of health of the colony. Small debris reflects good overwintering of bees, and large and moist one, the improper overwintering. Annual examination of debris allows the early detection of certain diseases and the possibility of potential therapeutic interventions such as (Chmielewski 1992):

- Debris – dead bees and the mentioned earlier ingredients are organic matter, which is the breeding ground and the substrate for the development of different species of mites as house dust mite, dried fruit mite, brown mite, storage mite, narrow mite, flour mite;
- The presence of fungal clods indicates the occurrence of tinea;
- Traces of feces are a sign of diarrhea in the colony, which is caused by nosemosis infection.

The inner and outer walls of the hive contaminated by feces indicate the presence of nosemosis in the colony. It should be done a study to determine the debris infection of bees with *Nosema* sp. spores.

Nosemosis of bees is well-known for beekeepers and veterinarians as a chronic of the honey bee (*Apis mellifera*) caused by intraspecific parasite *N. apis* specie – Eukaryota, Fungi, Microsporea (Gajda 2010). The parasite locates and develops in the cells of the midgut epithelium of workers, queens, and drones (Gliński and Rzedzicki 1983). *Nosema* spores are ingested along with food and water by adult honey bees. Symptoms of the disease include dysentery, increased winter loss of bees, reduced honey production, and shortened life span of the worker bees. These symptoms are most noticeable in the spring. The disease usually fades away in the summer and briefly returns again in the fall of the year (Reed 2010). Worker bees and queen bees have been found to become infected at similar rates when inoculated with *Nosema* spores (Thomas et al. 2004). The clinical symptoms appear only in severe course of disease (Table 1).

From a clinical point of view, nosemosis is a disease of older bees working outside the hive. Young bees, up to 15 days of age, were infected less often. The greatest severity of disease occurs in early spring – April, May (Glinski and Rzedzicki 1983). The disease spreads easily in a bee colony as bees licking the feces of sick bees infect themselves. Nosemosis causes death of bees (Marcinkowski 1994).

In the case of notice the symptoms of the disease it is proved it can be treated

TABLE 1. Summary of symptoms and changes occurring in nosemosis

Disease	Etiology and occurrence	Bees age	Clinical symptoms	Post-mortem lesions
Nosemosis	*Nosema apis* winter, early spring, spring	older bees	– loss of fly ability, abdominal distension, diarrhea – feces yellow loam color, grit-like consistency	midgut strongly inflated, pearly white

with many different preparations (Topolska et al. 2008, Nanetti 2009, Thrasyvoulou et al. 2009, Gajda 2010). At the low level of colonies contamination disease resolves spontaneously. In a time of generous food base and of radical replacement of source of infection – alive infected bees, it disappears (Tomaszewska 1995).

Results of apiary wintering is determined by the number of dead colonies during this period of time, as well as the state of health of those colonies which survived the wintertime. Taking care of health control and condition status of colonies during the overwintering it is recommended a debris composition and number of *Nosema* sp. spores inspection. Each year a number of colonies in the apiary cannot stand the wintering what is resulting in their weakening or death. On the strength of the colony during the wintering significant impact has breed of bees.

The aim of the experiment was to investigate and compare the components of the winter debris of two breeds of bees: Carniolan and Italian and microscopic tests of bees samples from the experimental debris for the presence of *Nosema* sp.

MATERIAL AND METHODS

The experiment was conducted on honey bee (*Apis mellifera*) Carniolan and Italian during the overwintering 2008/2009. The study was carried on 20 wintering bee colonies (10 of Carniolan bees and 10 of Italian bees) in the apiary of the Apiculture Division of Warsaw University of Life Sciences – SGGW. There were selected colonies of similar force, in the same types of hives (Wielkopolska bee hive), with the same number of frames and with a similar number of bees in colonies measured accordingly to the method described by Aimdorf and Gerig (1999).

The bottom boards of examined 20 hives were checked eight times during the wintering season. The measurements were taken at two-week intervals starting from 19 November 2008. Therefore, plastic pads were used, which have previously been properly fitted to the shape and dimensions of the bottom board of the hive so that they could be easily removed and replaced to and from hives (600 × 375 mm). Pads were placed in hives on bottom boards under the frames (5.11.2008) and all debris could fall freely directly to them. After each debris collection plastic pads were cleaned out and again placed on the bottom of the hive.

Winter debris was collected and the description of the components (such as bees, wax cappings, eggs, wax, pollen pellets, dark hardened fungal clods and fecal stains on the pads) measurement was made. Each test accurately determined the quantitative and qualitative composition of debris. To test the

significance of differences of the breeds of bees in each group of debris components (such as number of bees, number of eggs and number of wax cappings mean values) Student's t-test was used. This test applies to the statistics made on studied samples and is sufficient for used in the research groups size. Differences between the means in all groups were calculated at the 0.05 significance level (Olech and Wieczorek 2012).

There were also observed all abnormal changes that could be symptoms of diseases such as fecal spots or dark hardened fungal clods. Then, the tested material was packed into separate envelopes with pre-marked number of hives, the date and the information about the debris. Samples were stored in a freezer (at –20°C).

In the spring of 2009 microscopic examination was conducted (samples of bees from the experimental debris for the presence of *Nosema* sp.). *Nosema* detection method used in the study relied mainly on the procedures developed by Wilson and Ellis (1966), Cantwell (1970), Fingler et al. (1982), Kauko et al. (2002), Rogers et al. (2002), OIE (2004), Topolska and Hartwig (2005).

Examination was carried out as follows: to each sample were collected 30 bees to test it for the presence of *Nosema* sp. Abdomens were detached with tweezers and were homogenized with distilled water in a ratio of 1 ml per bee. A drop of the homogenate was transferred to a chamber used for counting blood cells (Fusch Rosenthal) and calculated the number of possible *Nosema* sp. spores in four small squares. In the case of infection number of *Nosema* sp. was calculated in a single bee according to the following formula:

10 squares 2 mm^3
1 square 0.2 mm^3
1/4 squares (4 – small squares) 0.05mm^3

$$X = \frac{\text{calculated number of spores} \times 10{,}000}{0.05} \quad (1)$$

It should be noted that the tested colonies were not treated for disease.

RESULTS AND DISCUSSION

In Carniolan bees hives on the bottom board was found on average less bees and wax cappings but more eggs than in Italian bee hives. Because of the wide variation in results (some hives had really small amounts of debris, but some hives were heavily littered) the standard deviations were high. There were not found significant differences between the means in any group (at the 0.05 significance level) of tested components between Carniolan and Italian race (Table 2).

In debris there was generally very small amount of wax itself, but pollen pellets was very common in almost every hive and in almost whole season (from November 2008 till March 2009). Especially in February when pollen pellets were found in all examined hives. Feces were observed only in three hives with various intensity and in one case the trace of faces did not indicated nosemosis infection (which was verified later by the test for *Nosema* sp. presence).

Fungal clods were found in January, in February and in March in four hives, but not too many of them. Generally it was more often observed in Italian bees hives (Table 3).

TABLE 2. The average number of some debris components in the whole wintering season in Carniolan and Italian bees hives

Apis mellifera breed	Value	Number of bees	Number of eggs	Number of wax cappings
Carniolan	mean	236.40	48.00	59.60
	SD	23.02	94.65	39.30
Italian	mean	326.50	33.00	61.75
	SD	110.41	34.30	19.00

TABLE 3. The results of the investigation of some debris components in Carniolan and Italian bees hives

Bee breed	Wax	Pollen pellets	Feces	Fungal clods
Carniolan	+	++++	+++	+
Italian	+	++++	–	++

Designations: (–) lack of component, (+) single presence, (++) double or few times presence, (+++) numerous, (++++) high intensity.

TABLE 4. Results of the dead bees samples tested for *Nosema* sp. in 2008/2009 season

Bee breed	Number of observations	The level of infection			
		– (N)	+ (S)	++ (M)	+++ (H)
Carniolan	80	69	3	2	6
Italian	80	76	0	1	3

Designations: (–) no spores, (+) single spores not in each field of vision, (++) single spores in each field of vision, (+++) numerous spores in each field of vision, (N) – not infected, (S) – slightly infected, (M) – moderately infected, (H) – heavily infected.

From that number only few hives showed *Nosema* sp. appearance. More often and more heavily infected were Carniolan bees (Table 4).

It was very interesting that at the beginning of the wintering the number of spores was high and from November till December even rose to the number of 16.6 millions of spores, it seemed to at the same level for some time but later on it seemed to fall and in March it reached about half a million of spores (Fig. 1).

CONCLUSIONS

1. Wax debris consisted of bees, eggs, wax cappings, crumbs of wax, pollen pellets, fecal spots and fungal clods of both bee breeds.
2. The presence of eggs in debris of both breeds testifies to the fact that in winter some queen bees are also lying eggs.
3. The presence of fecal spots in debris is sporadic in studied breeds but it is the sign of nosemosis.

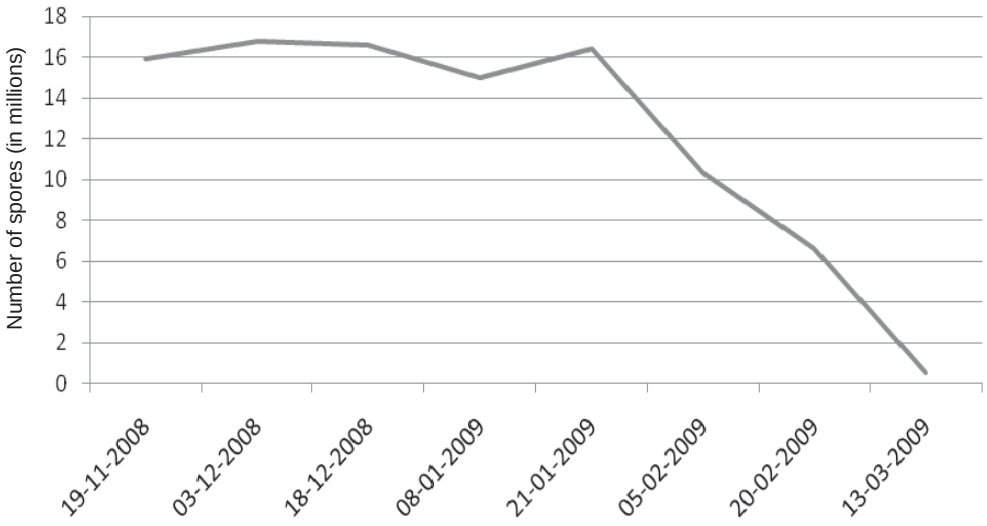

FIGURE 1. Number of spores of *Nosema* sp. in one of the Carniolan bees colony examined in 2008/2009 season

4. Microscopic examination confirmed the assumption that the appearance of the fecal spots was a symptom of *Nosema* sp. presence.
5. *Nosema* sp. infects bees of both breeds.
6. Lack of feces does not indicate the lack of nosemosis.

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Streszczenie: *Porównanie części składowych osypu zimowego oraz ilości spor Nosema sp. pszczół rasy kraińskiej i włoskiej*. Celem doświadczenia jest zbadanie i porównanie części składowych osypu zimowego pszczół dwóch ras kraińskiej i włoskiej oraz przeprowadzenie badań mikroskopowych prób pszczół z osypów doświadczalnych pod kontem występowania *Nosema* sp. Doświadczenie przeprowadzono na pszczole miodnej (*Apis mellifera*). Do badań użyto pszczół z 20 zimujących rodzin pszczelich w pasiece Pracowni Hodowli Owadów Użytkowych SGGW w Warszawie. Materiałem badawczym był osyp spadający w czasie zimy na dno ula, tzn. pszczoły, łuseczki woskowe, jaja, wosk, obnóża pyłkowe, ciemne stwardniałe grudki grzybicze oraz plamy kału na podkładkach. Doświadczenie przeprowadzono podczas zimowli 2008/2009. W okresie wiosennym 2009 roku przeprowadzono badania mikroskopowe (prób pszczół z osypów doświadczalnych na występowanie *Nosema* sp.). Obecność obnóży pyłkowych świadczy o obecności czerwiu w rodzinach obydwu ras. Obnóża pyłkowe pochodzą z pierzgi, którą pszczoły wykorzystują do karmienia czerwiu. Obecność jaj w osypie obydwu ras pszczół świadczy o tym, że w zimie niektóre matki też czerwią. Kał występuje sporadycznie i jest symptomem nosemy. Badania mikroskopowe potwierdziły przypuszczenie, że pojawienie się kału świadczy o obecności *Nosema* sp. *Nosema* sp. poraża pszczoły obydwu ras. Pszczoły rasy kraińskiej wykazują tendencje do samoleczenia. Brak kału u dwu ras pszczół nie wskazuje na brak nosemozy.

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