GENETIC, EPIGENETIC, MORPHOMETRIC AND BIOCHEMICAL IDENTIFICATION OF RESISTANCE AND SENSITIVITY TO TAU-FLUVALINATE *VARROA DESTRUCTOR* MITES FROM THE EASTERN POLISH APIARIES

Introduction

In 2000, Anderson and Trueman described four species of Varroa mites. *Varroa destructor* is the most common species worldwide.

Navajas and collaborators divided *Varroa destructor* mites into four basic haplogroups: Japanese, Korean, Chinese, and Vietnamese. The Korean haplogroup is the most dangerous.

It is known that mites parasitizing bees cause injuries.

In a review article "Biology and control of Varroa destructor", Rozenkranc reports that varroa mites pierce the integuments of the bee body with their teeth. The teeth were photographed for the first time by Ptaszyńska and Borsuk.

The puncture of bee's integument leads to hemolymph release, which next coagulates.

The clot is a convenient site for bacteria to penetrate the bee body, but this is not the major threat to bees.

The main risk to bees is posed by the salivary glands of Varroa destructor mites. After the bite, saliva containing bacteria and viruses enter the insect body and this is the primary cause of colony collapse disorder.

Mites parasitizing bees cause morphological deformations and physiological dysfunctions. Recently, mite control has become increasingly difficult, as no chemical agents are available, to which mites would not develop resistance.

Mites develop resistance at the level of the cuticle, where they increase the protective barrier of the chitin skeleton. Probably, although it has not been confirmed, they may degrade acaricides enzymatically using cytochrome P450.

Therefore, the aim of our investigations was to:

- determine the prevalence of resistance of *V. destructor* to tau-fluvalinate in eastern Poland,
- determine the existence of morphometric variations between resistance and sensitivity of *V. destructor*,
- determine the corelation between resistance of *V. destructor* and drugs,
- determine the mtDNA sequence variation,
- determine the proportion of methylated total DNA,
- determine the variability of surface proteins,
- an additional objective was to classify the mites occurring in Poland into haplogroups identified worldwide and to check whether the affinity is related to their resistance to tau-fluvalinate.

Materials and methods

We conducted our investigations for three years (from 2010 to 2012) and collected material from 82 apiaries in eastern Poland. In 36 apiaries only, we found at least 60 mites required to perform the Milani test for mite resistance to tau-fluvalinat.

From each apiary, we collected three combs with drone brood, which is preferred by the mites for their development.

As I have already mentioned, we assessed mite resistance using the Milani method.

We assessed the genetic variation on the basis of mitochondrial DNA for five sequenced gene fragments. However, only fragments of cytochrome oxidase subunit 1 genes with the length of 929 base pairs and cytochrome B with the length of 958 base pairs exhibited the highest variation. Therefore, we used them in our investigations.

The phylogenetic analysis was performed based on the cytochrome oxidase subunit 1 gene with the length of 929 base pairs using the MEGA 4.0.2 program

The surface proteolytic system of *V. destructor* mites was assessed with the method of Lowry, Schachterle and Pollack 1973, and Lee and Lin 1995.

The mites were measured on the dorsal and ventral side using a MultiScanBase v 14.02 computer program.

Results

During the three-year study, we found 2313 mites, out of which 380 were resistant to tau-fluvalinat, 1733 mites were sensitive to tau-fluvalinat, and 200 mites constituted the control group that was not subjected to the Milani tests and had no contact with tau-fluvalinat

Mites that were resistant to tau-fluvalinat appeared to be smaller than the sensitive mites.

The greatest number of resistant mites was found in 2012 and they made up almost 30%. It was alarming that there were apiaries with half or almost half of the mites were resistant to tau-fluvalinat.

During brood sampling in each apiary, we interviewed the beekeeper about treatment agents used in the previous beekeeping season/year and the length of treatment. This allowed us to determine the type of the active substance applied in the apiary and calculate its dose. This was the basis for assessment of the cross-resistance in *V. destructor* mites.

Mites exhibited the lowest resistance to the Polish drug Apiwarol, which contains amitraz as the active substance. This is probably related to the fact that the drug is administered by fumigation. The exposure time in a bee colony is short and lasts about 30 minutes. That is why V. destructor mites are not able to develop resistance in such a short time. This is also confirmed by the negative correlation between the per cent proportion of resistant mites and the drugs applied.

We found the greatest resistance of V. destructor mites to Bayvarol.

Tau-fluvalinat resistant mites had smaller quantities of protein on their body surfaces, but the protein exhibited higher activity. Therefore, we might postulate that the protein protects the mites from penetration of the active substance into the organism. It is possible that after application of acaricides the protein coagulates and forms a protein shell providing the mites with resistance.

Tau-fluvalinat resistant mites had the lowest proportion of methylated DNA. Therefore, we can claim that DNA methylation may be related to emergence of drug resistance in the parasites.

The RFLP-PCR analysis of the cytochrome oxidase subunit 1 gene with the length of 320 base pairs performed using two restriction enzymes Sac I and Xho I demonstrated that the mites belong to the *V. destructor* species. Enzyme Sac I recognised the restriction site and cleaved the fragment of cytochrome oxidase subunit 1 into two smaller fragments. In turn, enzyme Xho I did not recognise the restriction and failed to cleave the fragment of cytochrome oxidase subunit 1.

After sequencing the fragment of cytochrome oxidase subunit 1 with the length of 929 base pairs and comparing it with the NCBI database, we found that the Varroa destructor mites occurring in Poland belong to the Korean haplogroup 1 and Korean haplotype 1, Chinese haplotype 2 and 4.

After analysis of sequences of 4 gene fragments and the per cent proportion of resistant and sensitive mites, we did not find correlations between mite resistance to tau-fluvalinat and the haplogroup and haplotype.

Conclusions

1. All the *V. destructor* females from eastern Poland belong to the same first Korean haplogroup 1 (AmK1).
2. There was no correlation between *V. destructor* haplogroup and resistance to tau-fluvalinat.
3. The resistant *V. destructor* females displayed the lowest per cent proportion of total DNA methylation in comparison to the control and tau-fluvalinate sensitive *V. destructor* females.
4. Body size of *V. destructor* mites resistant to tau-fluvalinat was significantly smaller.
5. The resistant *V. destructor* females exhibited a lower concentration of protein on the body surface but the protein itself had a higher proteolytic activity. Hence, administration of acaricides, which act on mites through contact, may cause coagulation of proteins on mite body surface thereby producing a “protein shell” that prevents acaricides from penetration of the mite body and ensures their resistance.

Our team consists of: mycologists who deal with microsporidia, e.g. *Nosema* spp., a biochemist, beekeeping management, genetics, and is an inseminator of bee queens. We are an efficient team of bee specialists open to cooperation.