Praca oryginalna *Original paper*

Characteristics of north-eastern population of Varroa destructor resistant to synthetic pyrethroids*)

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Summary

The aim of the study was to discover whether strains of Varroa destructor resistant to synthetic pyrethroids can be found in north-eastern Poland and whether cross-resistance occurs in the north-eastern population of mites. The research was carried out from July 2009 to August 2011. Varroa mites from 79 apiaries from north-eastem Poland were examined. Varroa mites resistant to fluvalinate and flumethrin were found in one apiary situated near Olsztyn. These parasites showed an unusual ability to survive as compared to parasites from the other apiaries. In this apiary LC95 for fluvalinate was 5000 ppm, and for flumethrin, 100 ppm. Mites with a high risk of developing resistance to fluvalinate and flumethrin were discovered in two apiaries situated near Kwidzyn (about 100 km south of Gdańsk).

Keywords: Varroa destructor, resistant to synthetic pyrethroids, flumethrin, fluvalinate

Varroosis is a dangerous disease of bee colonies, affecting both brood and adult bees. In infested colonies, productivity decreases, and the colonies that are not treated against the disease die out in 3-4 years. A number of methods of controlling varroosis have been developed. Chemotherapy appears to be the most effective, but none of the medications is fully effective. Therefore, varroosis should be treated every year, which on the other hand entails the risk of drug resistance of *Varroa destructor*.

The highest effectiveness, i.e. over 95%, is shown by synthetic pyrethroids, and the drugs used in Poland in varroosis treatment are based mostly on their active substances. Among synthetic pyrethroids in use are fluvalinate and flumethrin. Beekeepers have recently been expressing dissatisfaction with the effectiveness of these substances, complaining about a large number of mites in the colonies in which the drugs were used. As a result, they have to carry out other procedures for controlling *Varroa destructor*, which generates additional costs in apiary management. Without extra measures, the colonies disappear in autumn and die out during wintering.

The ability of mites to develop drug resistance is believed to be at the root of a lower effectiveness of acaricides (10). The concept of resistance to natural toxins that is observed in animals is defined as the ability of the organism to tolerate a substance in toxic doses that may be lethal to most specimens in an ordinary population of the same species (21). It is genetically conditioned (6, 8, 13) and consists mostly in increased detoxification due to an increased activity of some enzymes (21). Developing resistance is enhanced by numerous factors: administering too small or too big doses of medications than the ones recommended by the manufacturer; keeping preparations in colonies for a time longer than recommended; using old, overused medications repeatedly; and using home-made preparations. Unfortunately, such mistakes are often made by beekeepers driven by economic reasons but oblivious to the fact that the short-term savings lead to greater long-term costs. These beekeepers frequently have insufficient knowledge on the subject. Resistance of *Varroa destructor* to acaricides may also be enhanced by the accumulation of their residues in apiary products, particularly in wax.

The first case of *Varroa* resistance to fluvalinate was noted in Sicily. Then, as a result of export, resistant mites spread to other European countries (20). In the meantime, strains of *Varroa destructor* resistant to other acaricides were found in a number of countries (15, 17). The development of drug resistance in the population

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of mites entails extremely unpleasant consequences for world beekeeping (5). In Italy, the losses attributed to the resistance of mites amounted to over 70% (2). In Poland, the first reports of reduced effectiveness of fluvalinate under field conditions appeared in 1996. Lipiński et al. (9) discovered strains characterized by a high risk of developing full resistance to fluvalinate in the Warmia region.

The aim of this study was to discover whether strains of *Varroa destructor* resistant to synthetic pyrethroids can be found in north-eastern Poland and whether cross-resistance occurs in the northeastern population of mites.

Material and methods

The research was carried out from July 2009 to August 2011 at the Apiculture Division of the University of Warmia and Mazury in Olsztyn, Poland. In the study, 79 apiaries from northeastern Poland were examined. The mites to be used in the experiment were collected mostly from young capped brood (stretched larva and pre-pupa stage). In some cases, when brood showed a low degree of infestation, females of *Varroa destructor* were taken from the pupa stage, but only dark brown females were chosen for the assay. For the purpose of testing drug resistance in a big apiary (of more than 50 colonies), brood samples were collected always from the colonies in the same apiary location. The combs with worker bee brood or drone brood scarcely covered with bees were collected from at least 8 randomly selected colonies, placed in a transporting box and immediately taken to the lab. Then the brood was incubated at a temperature of 35°C and humidity of 70%. Within 48 h from collecting the material, brood cells were opened in order to collect a number of adult *Varroa destructor* females necessary to test the mites' resistance to acaricides.

The testing of the mites' resistance to synthetic pyrethroids was carried out in accordance with a modified Milani's method (12). We used 16 glass capsules with tau fluvalinate (No CAS. 102851-06-9) in a concentration of 0.1-30 000 ppm, 12 capsules with flumethrin (No CAS. 69770-45-2) in a concentration of 0.2-1000 ppm, and 2 capsules without acaricides, necessary for control tests. Into each capsule we introduced 15 mites. In a few cases a smaller number of mites were introduced because of the difficulties in collecting mites; some mites also managed to escape from the capsules. The female *Varroa destructor* mites remained in the capsules for 6 h. After this time, they were placed in Petri dishes with three worker bee larvae from the cells capped 1-24 hours earlier. The mites were observed 6, 24 and 48 hours after having been placed in the capsules with acaricides in order to decide to which of the following categories they belonged: category I – alive and active; category II – alive but paralyzed; category III – dead. First, we tested concentrations of 200 ppm for fluvalinate and 20 ppm for flumethrin, which represented doses at which it is possible to distinguish a population resistant to these substances from a sensitive one (12, 13, 19). Next, depending on the results of the first test, we tested other concentrations until it was possible to determine a median lethal concentration of the acaricide – LC50, and a concentration lethal to 95% of the population – LC95.

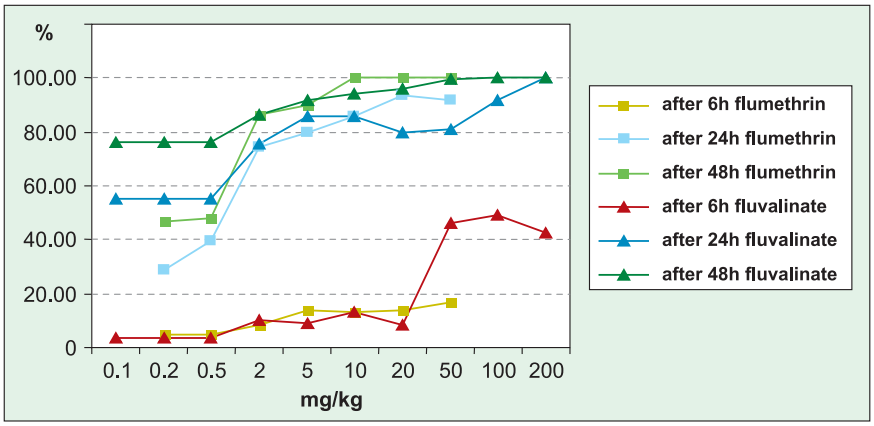

Fig. 1. Mortality of sensitive *Varroa* mites observed after 6, 24 and 48 h from placing them in capsules with pyrethroids in different concentrations

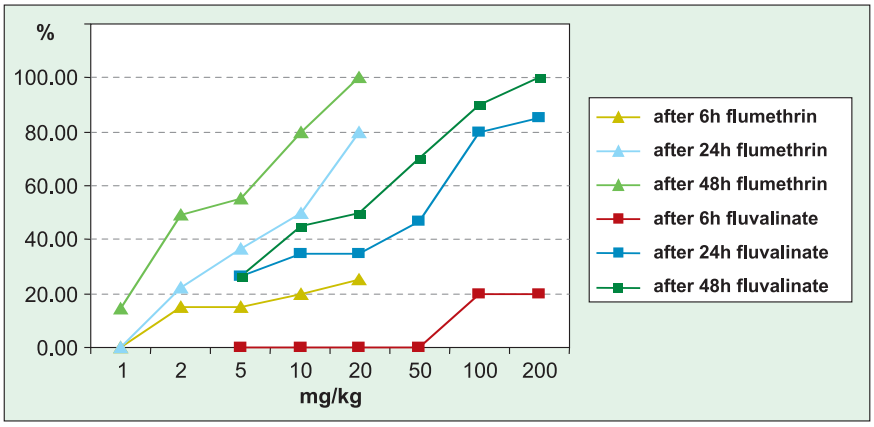

Fig. 2. Mortality of *Varroa* mites with a high risk of developing resistance, observed after 6, 24 and 48 hours from placing them in capsules with pyrethroids in different concentrations

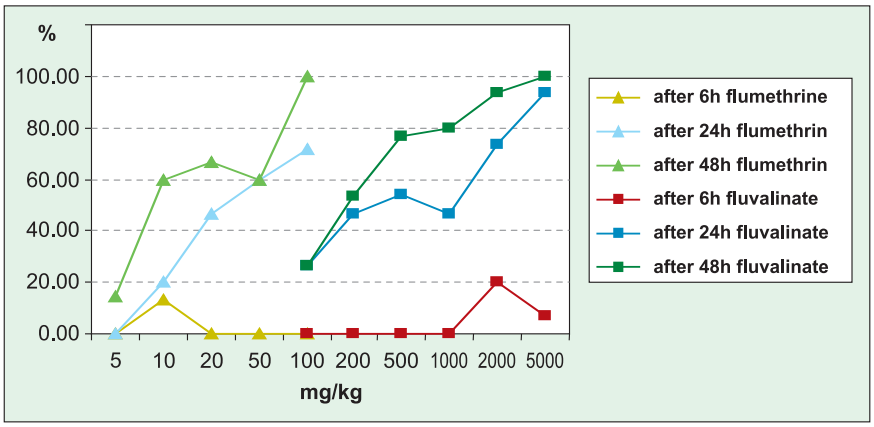

Fig. 3. Mortality of resistant *Varroa* mites observed after 6, 24 and 48 h from placing them in capsules with pyrethroids in different concentrations

Results and discussion

Out of the 79 apiaries, *Varroa* mites resistant to fluvalinate and flumethrin were found in one apiary situated near Olsztyn. These parasites showed an unusual ability to survive as compared to parasites from the other apiaries. In this apiary LC95 for fluvalinate was 5000 ppm, and for flumethrin, 100 ppm (Tab. 1). Mites with a high risk of developing resistance to fluvalinate and flumethrin were discovered in two apiaries situated near Kwidzyn (about 100 km south of Gdańsk).

The mortality rate of the parasites after 6 h from the moment of placing them in the capsules reached 50% at a fluvalinate concentration of 100 mg/kg only in the sensitive population (Fig. 1). In the remaining *Varroa* populations, mortality of the parasites after 6 h was 25% (Fig. 2 and 3). After 24 h mortality reached 100% at a f1uvalinate concentration of 200 mg/kg only in the sensitive population. The average mortality of the mites in the control group after 24 h from placing them in the capsules was 6.2%, and after 48 h, 11.07% (Fig. 4).

The degree of infestation in the bee samples collected from the apiaries with sensitive mites was 2.8%. A much higher degree of infestation was noted in bees from the apiaries with a high risk of developing resistance (20.5%) and from the apiary with resistant mites (37%).

The percentage of sealed brood cells infested with the mite in the samples from the apiaries with sensitive *Varroa* mites was 4.9%, and in the samples from the apiary with resistant mites, 42.5% (Tab. 2).

In the samples from the apiaries with a high risk of developing resistance, cells with 3 mature *Varroa destructor* females were not infrequent, and in the brood samples from the resistant apiary, cells with 4-5 mature females were often noted.

To control *Varroa destructor* mites, beekeepers from 73 apiaries used preparations based on amitraz and flumethrin. The average effectiveness of treating varroosis with amitraz was 93.6%, and with flumethrin, 88.8% (Tab. 3). In the remaining 6 apiaries, other preparations were used or no bee samples were acquired for the test. Extra procedures for controlling the mite were applied by 23% of beekeepers.

In the apiaries where mites with a high risk of developing resistance were found, only a preparation based on f1umethrin had been used for 4 subsequent years before the test. The distance between the two apiaries was 2 km, and the beekeepers strictly cooperated with each other (adding brood and bees to the colonies, as well as swarms and nuclei to the apiaries).

In the apiary with resistant mites, a preparation based on flumethrin was used for two subsequent years, and then no anti-varroa treatment was applied for a year. Next, a preparation with flumethrin was used again for three subsequent years. The same drugs were used for two years in a row, even though the manufacturer recommends abandoning the preparation after a single use.

Tab. 1. Median lethal concentration of synthetic pyrethroids – LC50, and concentration lethal to 95% of *Varroa destructor* population – LC95

V. destructor population	Fluvalinate (ppm)		Flumethrin (ppm)	
	LC 50 (min-max)	LC 95 (min-max)	LC 50 (min-max)	LC 95 (min-max)
Sensitive (76 apiaries)	0.9 (0.1-2)	29.2 (0.1-100)	0.5 (0.2-1)	6.7 (0.2-10)
Resistant (1 apiary)	200	5000	10	100
High risk of developing resistance (2 apiaries)	35	150	5	20

Tab. 2. Average infestation of bee samples and brood from apiaries with sensitive mites, with mites with a high risk of developing resistance, and with resistant mites

Infestation of apiary	Percentage of bees infested with parasite	Percentage of capped brood cells infested with parasite
Sensitive mites	2.8	4.9
Mites with a high risk of developing resistance	20.5	31.5
Resistant mites	37.0	42.5

Tab. 3. Effectiveness of using anti-varroa preparations in north-eastern Poland

Active substance	Number of apiaries	Average effectiveness (%)	Min-max (%)
Flumethrin	23	88.8	24-99.8
Amitraz	51	9.6	65.3-100

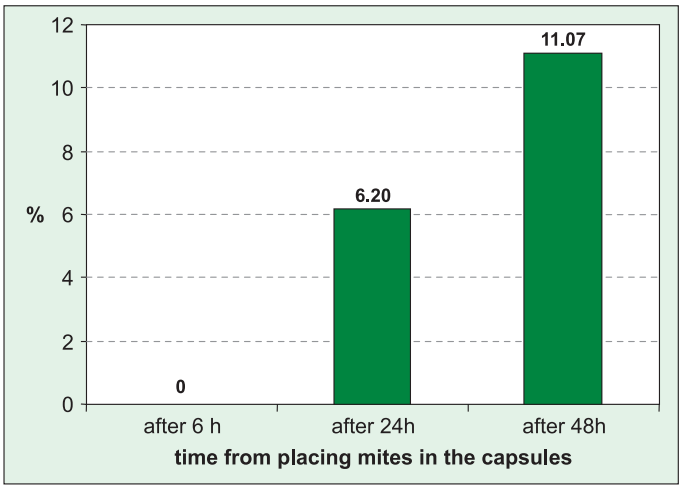

Fig. 4. Mortality of mites in control group

In all insensitive apiaries, after the test for mite resistance, an anti-varroa treatment based on preparations with amitraz was applied (average effectiveness: 98.8%).

Discussion

Arachnids develop resistance to acaricides particularly quickly (16). *Varroa destructor* mites, which belong to this group, have been proved to show resistance to many different substances: tetradifon (18), synthetic

pyrethroids (12), a mixture of sulfur and naphthalene (23), phosphoroorganic pesticides (15), and compounds of formic acid (10). This means that the world is well familiar with the problem. Many field and laboratory methods have been developed to effectively measure its extent (12, 14, 19, 20). As a result of such studies, different actions aimed at eliminating specimens resistant to the drugs used against them from the *Varroa destructor* population are carried out (4). Since the development of resistance is not an abrupt process – it can take up to 7 years (3) – an early detection of resistance to acaricides is of the utmost importance, as it may prevent losses in beekeeping. In our study, resistance to treatment in the apiary with resistant parasites, was detected too late. Replacing the active substance with another did not help because, after wintering, the beekeeper lost 75% of bee colonies. Possibly, the death of the colonies cured of varroosis had been caused by secondary infestations and overall weakness of worker bees growing in colonies highly infested with the *Varroa destructor* mite.

Since the level of drug resistance of *Varroa* in Europe has been assessed (1, 11, 20), it is recommended that it also be evaluated in Poland. Even if parasites in Poland have not developed such a defense mechanism against acaricides by themselves, it is highly probable that resistant mites have been introduced to the local population with imported honeybee queens or bee packets from different parts of Europe. Migratory apiary management constitutes an additional factor contributing to the proliferation of resistant *Varroa* mites. Drug resistance is in turn preserved by the above-mentioned mistakes made by vets and beekeepers when treating varroosis. It is almost certain that the drug resistance discovered in the population of *Varroa destructor* near Olsztyn had been to a great extent enhanced by wrong procedures undertaken by the beekeeper.

In Poland, the problem of the resistance of *Varroa destructor* to contact acaricides has been studied by few researchers. Their findings, however, have sufficiently demonstrated that the resistance also occurs in Poland (7-9). Therefore, it is recommended that the extent of the problem be assessed by meticulous laboratory tests. The problem of ineffective treatment in infested bee colonies has been attracting increasing interest in Poland, as well as all over Europe (the Bayer company has organized two conferences devoted to this issue), since the resulting losses in beekeeping lead to further losses in agriculture, caused by the fact that the honeybee, attacked by the mite, is an irreplaceable pollinator. In Poland, the losses attributed to ineffective pollination of entomophiłous crops are estimated at PLN 80 m. (22). The EU has also took interest in the problem and granted funds to diagnose the causes of and assess the losses generated by the mass dying of bee colonies in the world, as part of the COLLOS program carried out in cooperation with Canada, China, Puerto Rico and the USA. The increasing drug resistance of *Varroa destructor* is also mentioned as one of the causes.

Conclusions

The results of our study show that in northeastem Poland there are populations of mites resistant to treatment, as well as populations with a high risk of developing resistance. Cross-resistance was also observed. This finding must not be ignored. A major sign of a decreasing sensitivity of *Varroa destructor* mites to synthetic pyrethroids is a high degree (over 20%) of infestation with this parasite in bees and brood.

When resistance is discovered, replacing one anti-varroa preparation with another may not save the apiary. Research should be continued to monitor the risk of parasites resistant to acaricides appearing in the local population, which would help prevent an abrupt loss of bee colonies in individual regions of north-eastern Poland.

References

1. *Colin M. E., Vandame R., Jourdan P., Dipasquale S.*: Fluvalinate resistance of Varroa jacobsoni Oudemans (Acari: Varroidae) in Mediterranean apiaries of France. Apidologie 1997, 28, 329-472.
2. *Colombo M. M.*: Don't abuse Apistan. Bee Culture 1995, 122, 590-593.
3. *Eischen F.*: Varroa resistance to fluvalinate. Am. Bee J. 1995, 10, 815-816.
4. *Elzen P. J., Baxter J. R., Westervelt D., Causey D., Randall C., Cuttis L., Wilson W. T.*: Acaricide rotation plan for control of Varroa. Am. Bee J. 2000, 141 (6), 412-416.
5. *Goodwin R. M., Taylor M. A., McBrydie H. M., Cox H. M.*: Base levels of resistance to common control compounds by a New Zealand population of Varroa destructor. New Zealand J. Crop and Horticult. Sci. 2005, 33, 347-352.
6. *Hemingway J., Ranson H.*: Insecticide resistance in insects vectors of human disease. Ann. Rev. Entomol. 2000, 45, 371-391.
7. *Lipiński Z.*: Badania oporności Varroa destructor na pyretroidy. Pasieka 2006, 5, 28-30.
8. *Lipiński Z., Szubstarski J.*: Resistance of Varroa destructor to most commonly used synthetic acaricides. Pol. J. Vet. Sci. 2007, 10, 289-294.
9. *Lipiński Z., Żółtowska K., Łopieńska E.*: Preliminary evidence of associating oxidative stress in honey bee drone brood with Varroa destructor. J. Apic. Res. 2005, 44, 126-127.
10. *Mathieu L., Faucon J. P.*: Changes In the response time for Varro jacobsoni expose to amitraz. J. Apic. Res. 2000, 39, 155-158.
11. *Milani N.*: The resistance of Varroa jacobsoni Oud. to acaricides. Apidologie 1999, 30, 229-234.
12. *Milani N.*: The resistance of Varroa jacobsoni Oud. to pyrethroids a laboratory assay. Apidologie 1995, 26, 415-429.
13. *Milani N.*: The resistance to chemotherapy in parasites and pathogens of the honeybee. Proc. Euroconference MOMEDITO, Kralupy near Prague 2000, 17-19 October 2000, p. 117-131.
14. *Milani N., Della Vedova G.*: Decline in the proportion of mites resistant to fluvalinate in a population of Varroa destructor not treated with pyrethroids. Apidologie 2002, 33, 417-422.
15. *Milani N., Della Vedova G.*: Determination of the LC50 in the mite Varroa jacobsoni of the active substances in Perizin and Cekafix. Apidologie 1996, 26, 67-72.
16. *Möbus B., Connor L.*: The Varroa hand book: biology and control Northern Bee Books; Mytholmroyd, UK 1988, 52 pp.
17. *Ritter W., Roth H.*: Experiments with mite resistance to varroacidal substances in the laboratory, [in:] Cavalloro R. (ed.), European research on varroatosis control. Proc. Meet. E.C. Experts Group, Bad Homburg, October 1986, Balkema, Rotterdam 1988, 157-160.
18. *Smirnov A. M.*: Research results obtained in USSR concerning aetiology, pathogens, epizootiology, diagnosis and control of Varroadisease in bees. Apiacta 1978, 13, 149-162.
19. *Thompson H., Brown M., Ball R., Bew M.*: First report of Varroa destructor resistance to pyrethroids in the UK. Apidologie 2002, 33, 357-366.
20. *Trouiller J.*: Monitoring of Varroa jacobsoni resistance to pyrethroids in western Europe. Apidologie 1998, 28, 537-546.
21. *Watkins M.*: Resistance and its relevance to beekeeping. Bee World 1996, 78 (1), 15-22.
22. *Wilde J.*: Ochrona zdrowia pszczół, a problem oporności Varroa destructor na syntetyczne akarycydy kontaktowe. Mat. symp. nauk. firma Bayer. Tomaszów Maz. 9-10.01.2008, p. 2-7.
23. *Wongsiri S., Tangkanasing P., Sylvester H. A.*: Mites, pests and beekeeping with Apis mellifera in Thailand. Am. Bee J. 1987, 127, 500-503.

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