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Detection of the high risk pyrethroid resistant *Varroa destructor* mites in apiaries of the Warmia-Mazury province in Poland

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ABSTRACT. **Background**. The aim of our current study was to investigate the possible occurence of pyrethroid (tau-fluvalinate) resistant *Varroa* mites infestations in 24 randomly chosen apiaries of Warmia-Mazury province of north-east Poland. **Methods**. The methodology used for the analysis of resistant *Varroa* strains strictly followed the protocol described by Milani [6, 14]. **Results**. We identified 3 apiaries that were infested with high risk pyrethroid resistance mites and a further 9 apiaries that were free from this resitance. The brood samples collected from the remaining apiaries did not contain sufficient numbers of parasites to enable us to properly perform the assay. **Conclusions**. Our finding that 25% of the tested brood samples showed a high risk of fully pyrethroid resistant *Varroa* mite contamination indicates that resistant *Varroa* may become wide spread in apiaries in Poland. Interestingly these high risk resistant mites were found in honeybee colonies with low levels of *Varroa* infestation, with an average rate of 2.16%. We also discuss the role of amitraz (amidine) in the phenomenon of *Varroa* resistance to pyrethroids.

Key words: acaricide resistance, amitraz, *Apis mellifera*, honeybee, pyrethroid, *Varroa destructor*.

Introduction

The *Varroa destructor* [1] mite is potentially the main threatening parasite for honeybee colonies of *Apis mellifera* (L.) worldwide. If an infested colony is not properly treated, it will generally die within a few years [2–4]. According to the report of De Jong et al. [5], mortality from *V. destructor* can reach 100% within 2 years if mite control measures are not implemented.

It was not until the end of the 1980s that satisfactory control procedures for the *Varroa* mite were available using pyrethroid based acaricides such as tau-fluvalinate. These agents have a high efficacy against *Varroa* mites and low toxicity toward honeybees. However since the middle of the past decade, resistance to pyrethroids (particularly tau-fluvalinate) has arisen in the *Varroa* mites in different parts of the world including Europe [6, 7] and the United States [8, 9]. It is of particular concern also that these mites can develop cross resistance both to fluvalinate and two closely related pyrethroids: flumethrin and acrinathrin [10].

The median lethal concentrations (LC_{50}) of mites originating from areas where treatments with fluvalinate are no longer effective was found to be about 25–50 fold higher than that of susceptible mites. Likwise, the LC_{50} of flumethrin and acrinathrin against mites surviving fluvalinate treatments increased 10–60 fold [6]. As result of these phenomena, heavy losses of honeybee colonies have been reported in a number of different European countries [10–12].

It is note-worthy that the term resistance is taken to mean „the ability of an organism to tolerate toxic doses of a substance that would be lethal to the majority of individuals in a normal population of the same species" [13]. Resistance to pesticides is a natural phenomenon which involves rare mutations within a few individuals followed by the selection, of these resistant individuals and their offspring in presence of the pesticide. Hence the greater the number of generations that are reared in presence of an pesticide, the higher the proportion of resistant populations will be [14]. According to this study of Milani also the appearance of resistant strain is actually rare, and the main concern is more likely to be the spread of resistant strains.

It is generally difficult to detect the presence of resistance in the absence of an effective laboratory assay [14]. Hence, several laboratory methodologies have now been developed to test resistance to pyrethroids [12, 14–17]. Among these tests, Milani [14] has developed an assay which is now very popular, due to it accuracy and ease of use. This test is based in a controlled contact of mites with a known discriminating concentration of tau-flivalinate in glass capsules.

The detection of strains of *Varroa destructor* that are resistant to fluvalinate, were initially reported in Italy as a result of the availability of these laboratory tests and field trials [18, 19]. In 1995, mite populations exhibiting resistance to fluvalinate were also detected in south-eastern France and Switzerland [13]. The first evidence for the presence of pyrethroid resistant *Varroa* in Poland was provided by Londzin and Śledziński [20], who found, that the efficacy of treatment of the honeybee colonies with tau-fluvalinate on four occasions between 1991-94 and twice between 1993–94 was 66.4% and 99.2% respectively.

Given that there is now an increased use of flumethrine in Polish apiaries and also additional applications treatments with other acaricides, mostly amitraz, using smoke (Apivarol) and strip applications (Biovar, Apitraz), we decided to study the current incidence of pyrethroid resistant *Varroa* in apiaries of north Poland.

Material and methods

Testing of the resistance of *Varroa* mites to tau-fluvalinate was based on naturally infested brood samples, which were collected from 12 randomly chosen apiaries in different counties of the Warmia-Mazury province in north Poland. Each sample of a living *Varroa* infested drone and/or worker brood (consisting of about 3500 worker cells or 3000 drone brood cells, respectively), was collected from one to three colonies in each apiary. The smallest apiary that we sampled comprised 5 hives (Table 1, no. 3) whereas the biggest consisted of 50 hives and formed part of complex apiary of about 2000 hives (Table 1 no. 16). These investigations were carried out in the late spring and summer 2006.

The samples were delivered to our laboratory by road transport and analysed within 2 days of sampling. To lessen the risk of testing isolated mites with a low fitness we maintained them for 24 hours in an incubator (32 ± 1°C, 75% RH). Each sample of combs for drone or worker broods was uncapped. Mites that were isolated from comb samples at a population of less than 65 individuals were not analysed. Furthermore, only mites showing normal movement behaviours were used in these tests. Additionally, information was also collected regarding the dates upon which *Varroa* treatments occured in each apiary during the years 2001–2005.

The methodology used for the analysis of *Varroa* resistant strains strictly followed the protocol described by Milani [6, 14]. The authors of this study received training in this methodology during their visit to the Department of Biologia Applicata at Udine University in May 2006.

Briefly, collected *Varroa* mites (n=55) were analysed for their susceptibility to tau-fluvalinate (death or paralysis) at the discriminating concentration of 200 mg/kg paraffin (the concentration at which 99.7% of the susceptible mites are killed) with the control experiment performed at 0 mg/kg paraffin (n=10). When mortality in the control experiments were higher than 5%, the sample was discarded.

The resistance to tau-fluvalinate was assessed in *Varroa* according to a three step scale where survival at the level of 1–5% means lack of resistance, at the level of 5–10% means a high risk of resistance, and a survival rate higher than 10% indicates full resistance to tau-fluvalinate and other pyrethroids.

Results

The infestation rate of 24 worker and drone brood samples by *Varroa* mites was found to have reached an average level of 2.3%, which allow the minimal number of 65 *Varroa* mites to be sampled and ensure the correct processing of the assay, in 12 cases only.

We found that three brood samples (Table 1, nos 1,7,10) contained mites, showing a high risk of pyrethroid resistance at levels of 6.2%, 7.2% and 5.4%, respectively (Table 1). The mites from the remaining 8 apiaries did not show any resistance —

as the survival rates of the mites from these samples ranged from 0 to 4.9%.

In two of the three apiaries that we recognized as high risk for pyrethroide resistance (6.2 and 7.2% mite survival), the beekeepers had continuously used Bayvarol since 2001 (Table 1). In the third high risk case (5.4% survival) the Bayvarol treatment (years 2004–2005) had only been used on colonies that had been three times treated (years 2001–2003) with amitraz in smoking tablets (Apiwarol).

Interestingly the 4.9% level of resistance to fluvalinate in mites from apiary no 16, manifested after five each years of continuous treatment with amitraz in smoking tablets.

Discussion

We observed from our current analysis, that out of 12 Polish apiaries under investigations for infestation by perethroid resistant *Varroa* mites, three showed high survival rates in our laboratatory assay (7.2%, 6.2% and 5.4% respectively). This indicates that fully pyrethroid resistant *Varroa* mites could emerge throughout Poland, as it was suspected by Londzin and Śledziński [20]. This possibility is further strenghtened by the fact that full resistance to pyrethroids is becoming more wide spread in *Varoa* throughout Europe [6, 7].

In an Austrian study, out of the 25 samples analysed, 12 presented with resistance levels ranging from 7–27%. In another report from Belgium, 22 samples were analysed and 13 showed resistance figures between 1.7–20%. In France, out of the 50 samples analysed, 26 showed resistances of 2 to 100%. In Hungary, the only sample analysed presented a resistance level of 40%, whereas in study from Sardinia, which is probably the first location where *Varroa* resistance was reported out of the 14 samples investigated, 13 presented resistances ranging 3–96%, reviewed by Trouiller [**7**].

It is significant also from our current data that out of the three apiaries investigated, two were found to be at high risk of developing fully pyretroid resistant *Varroa*, even though they had undergone five years of flumethrine (Table 1). This confirms the hypothesis of Eischen [21], that the emergence of full resistance to pyretroids in mites usually requires 6–7 years of constant contact with this acaricide and is consistent with the earlier finding that a period of four years is sufficient for the development of such a levels of resistance [20].

In this context, it is of further significance that our current observations show that a high risk of developing resistant mites appears to coincide with colonies harbouring relatively low levels of *Varroa* infestation rate. This is mainly due to previous exclusion of capped drone brood and the autumm

Table 1. Estimation of the development of pyrethroid resistant *Varroa* mites in apiaries with a 5 year history of treatment with different acaricides.

Sample number	Resistant %	Acaricides used in years 2001–2005	No of sample	Resistant %	Acaricides used in years 2001–2005
1	**6.2**	**FL FL FL FL FL**	13	(—)	AT AT AT AS AS
2	(—)	AT AT AT AT AT	14	(—)	AT AT AT AS AS
3	(—)	AT FL FL FL AT	15	(—)	AT AT AT FL FL
4	2.8	**FL FL FL FL FL**	16	4.9	AT AT AT AT AT
5	2.3	AT AT AT AT FL	17	0	AT AC AC AS FL
6	(—)	FL AT AT FL AT	18	0	BF AT AT AT AS
7	**7.2**	**FL FL FL FL FL**	19	(—)	AT FL FL FL AT
8	(—)	AT AT FL FL FL	20	(—)	**FL FL FL FL FL**
9	0	AT AT AS AS FL	21	0.9	AT AT AT AT AT
10	**5.4**	**AT AT AT FL FL**	22	(—)	**FL FL FL FL FL**
11	0	FL BF AT AS AS	23	(—)	AT FL FL FL FL
12	0	BF BF AS AS FL	24	(—)	**FL FL FL FL FL**

(—) — insufficient number of *Varroa*, AC — acrinathrin (Gabon), AS — amitraz in strips (Biovar), AT — amitraz in fumigant treatments to eridicate the mites, but is an important concern from an practical point of view, as beekeepers will usually not associate the presence of *Varroa* resistance with the use of acaricides and with low levels of infestation by these parasites.

Interestingly the lack of mites that are resistant to fluvalinate in sample no 4 (Table 1), despite a 5 year course of flumethrine treatment seems to suggest, that resistance to pyrethroids, like resistance to amitraz is emerging in *Varroa* populations from several different locations [22].

Moreover, the indication of a high risk of pyretroid resistant mites at levels of 5.4% in apiary no 10, after 3 year use of amitraz and 2 year use of flumethrin, and a 4.9% level of resistance in apiary no 16, after a 5 year use of amitraz (Table 1), seems to confirm the findings of Elzen et al. [23] that "amitraz can facilitate the selection of pyrethroid resistant *Varroa* mites" to such an extent that it is not "usefull for control of fluvalinate resistant *Varroa* mites" [24].

In this regard also it has been shown that: (1) the detoxication abilities of mites can be selected both by pyrethroids [10, 13, 25] and by amitraz [12, 26] and (2) the biological action of amitraz is due to its sublethal effects, rather than to a direct lethality [27].

Conclusions

Our current observations show that out of the 12 apiaries under study from north Poland 3 are at high risk of developing pyrethroid resistant *Varroa* mites, indicating that full resistance to pyrethroids in these parasites may become wide spread in apiaries in Poland. A high risk of infestation with pyrethroid resistant mites can also be associated with honeybee colonies harbouring low infestation rates. Further investigations are also needed regarding the role of amitraz in contributing to this phenomenon.

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