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PP-085 [Bee Health]

Single-Nucleotide Polymorphisms (SNPs) in the ORβ-2R-L Gene of French and Spanish Varroa Mite Populations Are Not Directly Linked to Amitraz-Resistant Phenotypes

Authors: Ulrike Marsky[1], Abderrahim Hammaidi[1], Rémi Padé[1]
[1]Véto-pharma, 12/14 Rue de la Croix-Martre, 91120 Palaiseau, France

INTRODUCTION

Amitraz resistance has been linked to **Single-Nucleotide Polymorphisms (SNPs) in the ORβ-2R-L gene**, suggesting a relatively simple genetic mechanism leading to a form of target site resistance. However, as highlighted by *Marsky et al.* (2024) in *Insects*, the resistance phenotype **may involve more complex, multifactorial processes beyond SNPs alone**, warranting deeper genomic and environmental investigation.

Here, we present data:

1. Investigating bioassay protocols

2. Supporting the hypothesis that SNPs in the varroa mite ORβ-2R-L gene alone can likely not explain the development of amitraz resistance.

These data were collected in an experimental (preliminary) bioassay test in Spain with mites samples from an organic apiary, and a bioassay test in France after reduced field efficacy had been observed on the sampled honey bee colonies.

METHODOLOGY

In autumn 2022, bioassays were conducted in France and Spain to assess amitraz resistance in *Varroa destructor*. In Spain, 12 colonies from an organic apiary near Léon were divided into three exposure groups (4, 6, and 8 hours) using 3.7×2.2 cm Apivar® strip pieces fixed in Varroa EasyCheck® baskets, with ambient temperature at 21 °C. In France, 200 bees from each of 7 colonies were placed in honey jars with mesh lids and exposed to 1×2 cm Apivar® strip pieces for 6 hours at 25–30 °C. Post-exposure, 52 mites from three of the sampled hives in France (26 survivors, 26 perished) and 50 mites from the five hives in Spain were genotyped to :

1. Determine if any Single-Nucleotide Polymorphisms (SNPs) potentially associated with amitraz resistance were present in the ORβ-2R-L gene.

2. Investigate correlations between sensitivity towards amitraz exposure and SNP expression in the *ORβ-2R-L* gene.

Figure 1: Varroa EasyCheck® devices including the bee samples and varroa mites.

Figure 2: Apivar® strip (including the V-shaped opening) attached to the white basket of the Varroa EasyCheck® device.

RESULTS FROM SPAIN

In the bioassays conducted in Spain, a total of 12 out of 204 tested varroa mites from an organic apiary survived the bioassay exposure to amitraz (Figure 3). We identified a previously unknown mutation in the *ORβ-2R-L* gene of these mites from the Léon region: the mutation T868C (a substitution of T to C at position 868). None of the mutations previously described in the literature about amitraz resistance in varroa mites (Hernández Rodríguez et al. (2022): substitution of A to G at position 260 (A260G) and substitution of T to C at position 643 (T643C)) were detected in these mites (Figure 4). Varroa mites which survived the exposure towards amitraz in the bioassay did not present any specific mutation more frequently compared to the mites which fell during the incubation period (mites from the M1 group).

Figure 3 - Spain: Bioassay Results

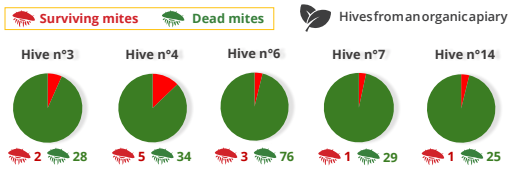

Figure 4 - Spain: Genotyping Results

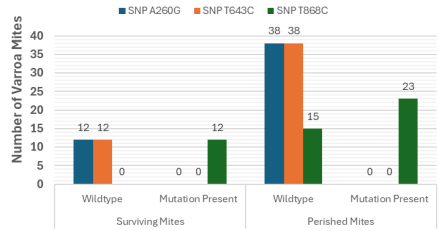

Figure 2: 50 mites from Spain (12 survivors, 38 perished) were genotyped. The Single Nucleotide Polymorphisms (SNPs) first identified in North America (Y215H) and France (Mutation N87S) were not present in these mites - independent of their sensitivity towards amitraz. A previously unknown mutation at location T868C was identified in all of the 12 mites which had previously survived the exposure to amitraz in a bioassay and in 23 of the 38 mites that had perished during the amitraz exposure.

RESULTS FROM FRANCE

In the bioassays conducted in France, a total of 49 out of 125 tested varroa mites survived the bioassay exposure to amitraz (See Figure 5). This corresponds to 39% of the tested varroa mites surviving the exposure towards amitraz and therefore being classified as "resistant". In a later genotypic analysis (Figure 6) of the mites' *ORβ-2R-L* gene, we identified 3 distinct mutations in these mites: the mutation described by Hernandez Rodriguez (A260G) (about 36.5% of mites), and two mutations already identified in previous unpublished analyses of French varroa mites: C248T and CA344-345AT. Neither the "American mutation" (T643C), nor the "Spanish mutation" (T868C) was identified within the part of the gene *ORβ-2R-L* analyzed for these mites. None of the mutations identified here occurred more or less frequently in resistant mites which survived the bioassay exposure towards amitraz.

Figure 5 - France: Bioassay Results

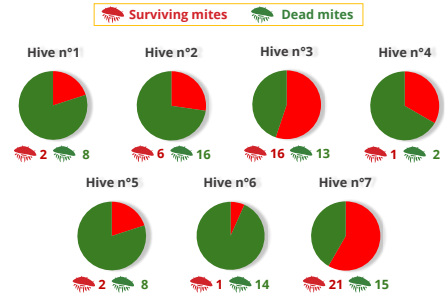

Figure 6 - France: Genotyping Results

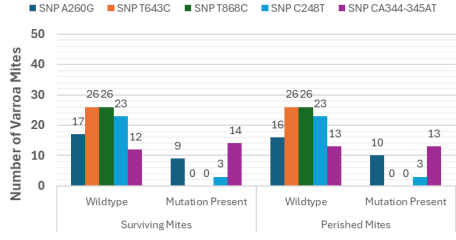

Figure 4: 52 mites from France (26 survivors, 26 perished) were genotyped. The Single Nucleotide Polymorphisms (SNPs) first identified in North America, T643C (or Y215H), and Spain (SNP T868C) were not present in these mites - independent of their sensitivity towards amitraz. The mutation N87S (SNP A260G), described by Hernández Rodríguez et al. (2022), was present in mites which survived amitraz exposure and mites that perished during the bioassays in the same frequency. Two previously unknown mutations were found at positions C248T and C344A-A345T. The frequency of these mutations did not differ between surviving mites and perished mites.

CONCLUSION

1. **No direct association was found between individual SNPs in the mites' ORβ-2R-L gene and their phenotype in French and Spanish Varroa mite populations.**
2. **Multiple SNPs were identified, including a novel T868C mutation in Spanish mites from an organic apiary that has not been treated with amitraz for several years.**
3. **None of the mutations was present predominantly in mites phenotypically resistant to amitraz.**
4. **Results indicate that amitraz resistance likely involves complex mechanisms, possibly extending beyond target site resistance.**
5. **Further research is recommended to investigate multifactorial genetic and environmental factors contributing to resistance.**

REFERENCES

Marsky, Ulrike, et al. "Amitraz resistance in French Varroa mite populations—more complex than a single-nucleotide polymorphism." Insects 15.6 (2024): 390.

Hernández-Rodríguez, Carmen Sara, et al. "Resistance to amitraz in the parasitic honey bee mite Varroa destructor is associated with mutations in the β-adrenergic-like octopamine receptor." Journal of Pest Science 95.3 (2022): 1179-1195.

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Ulrike Marsky, Abderrahim Hammaidi, Rémi Padé
Department of Research and Development, Véto-pharma, Palaiseau, France

For decades, amitraz has been utilized by beekeepers globally as an effective control method for varroa mite (Varroa destructor) infestations in honey bee (Apis mellifera) colonies. Reports of amitraz resistance, mainly from commercial beekeeping operations in France and North America, have recently been linked to the identification of single-nucleotide polymorphisms (SNPs) in the ORβ-2R-L gene in the mites' genome. Here, we present data collected from apiaries from the AURA region in France and the Valencia region in Spain in 2022. Bioassay ("Pettis test") outcomes, which detected the susceptibility of individual varroa mites towards amitraz, were recorded, and each mite was classified as either "sensitive" or "resistant". We later genotyped 92 of these individual mites which had previously been categorized in accordance with their phenotypic response towards amitraz. The genetic analysis focused on the β–adrenergic-like octopamine receptor, which is considered the primary target for amitraz in varroa mites. Particular attention was given to a SNP at position 260 of the ORβ-2R-L gene, which had previously been described by Hernández Rodríguez et al. (2021). In addition, we identified a novel SNP at position 868 of the ORβ-2R-L gene that was only identified in the Spanish mite sample. We also identified a mutation at positions 344-355 in the same gene, which occurred only in the French mite sample. Our results confirm our own previous results published in 2024, suggesting that there is no direct link between the SNP at position 260 of the ORβ-2R-L gene alone and amitraz resistance development in varroa mites. Furthermore, our genetic analyses suggest that the SNP at position 868 of the ORβ-2R-L gene as well as the mutation at positions 344-345 in the same gene are not directly associated with amitraz resistance development either. These results reinforce the higher level of complexity involved in the development in amitraz resistance compared with pyrethroid or coumaphos resistance in varroa mites. They also align with recent publications suggesting a higher genetic variability in the varroa mite genome than previously expected due to the mites' incestuous reproductive system.