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PP-165 [Bee Health]

Exploring Pyrethroid and Amitraz Resistance in *Varroa destructor* Mites from Honeybee (*Apis mellifera*) Colonies in Portugal

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Introduction

Varroa destructor, an ectoparasitic mite of honey bees (*Apis mellifera*), poses a significant threat to apiculture by causing varroosis and transmitting viruses, including the deformed wing virus. This mite can be controlled by the use of synthetic or organic acaricides. However, the excessive and repeated use of acaricides has led to resistance in mite populations.

Resistance to pyrethroids, is linked to mutations at positions 925 (L925V, L925I, L925M) and 918 (M918L) of the voltage-gated sodium channel (VGSC) gene. For amitraz, resistance is linked to mutations in the Octβ2R gene, at position 87 (N87S), 215 (Y215H), 290 (F290L), and 337 (Y337F).

Objectives

- Detect the presence of resistance alleles to pyrethroids at positions 918 and 925 in Portugal
- Detect the presence of alleles associated with resistance to amitraz
- Map the national distribution of these resistance alleles

Materials & Methods

Sampling: Mites were collected across Portugal in 2024/2025.

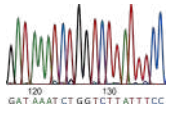
82 apiaries **(Pyrethroids)**
27 apiaries **(Amitraz)**

Varroa destructor analyzed
245 samples **(Pyrethroids)**
59 samples **(Amitraz)**

Laboratory

✓ **DNA Extraction**
- From a single *Varroa destructor*

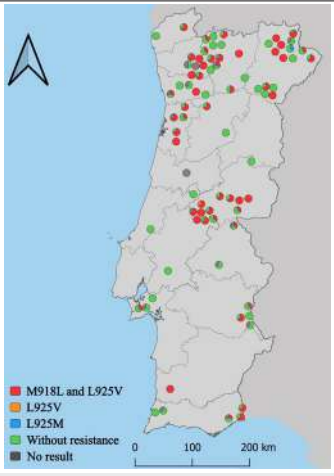
✓ **PCR**
Two **locus-specific primers** were used:
- One targeting known mutations associated with **pyrethroid resistance;**
- One targeting mutations linked to **amitraz resistance (Y215, F290 and Y337)**.



✓ **Sanger Sequencing**
- Sequencing was performed by Macrogen Europe (Madrid, Spain);
- Chromatograms were analyzed and aligned using (Bioedit and MEGA)

Results & Discussion

Apiary-level results: spatial distribution of pyrethroid resistance in Portugal.



Spatial distribution of apiaries and amitraz samples.

Pyrethroids: Of the 245 samples analyzed, 20 (8%) did not yield valid results. Of the remaining samples, 107 (43.7%) showed the simultaneous presence of the L925V mutation — typical in Europe — and the M918L mutation, initially identified in the Valencian Community, Spain. Only one sample (0.4%) presented the L925V mutation alone, and another (0.4%) revealed the L925M mutation, originally described in the United States. On the other hand, 116 samples (47.3%) did not exhibit any mutation associated with pyrethroid resistance and were therefore considered susceptible, of which 3.4% were heterozygous.

Amitraz: Remarkably, 98.3% of the 59 amitraz samples (58 samples) carried the Spanish F290L mutation, with just one sample (1.7%).
The Y215 mutation (USA) and the Turkish Y337 mutation were not detected in any of the samples. In this study, no samples were analyzed for the N87 position using a primer targeting this mutation. This mutation has been previously reported in other European countries and will require further investigation in future analyses.

Conclusion

Regarding pyrethroids, the most common mutation among the samples was the M918L + L925V combination, which is expected given Portugal proximity to Spain. Regarding amitraz, the recent Spanish F290L mutation was detected in 98.3% of the samples, and its high prevalence should be further investigated.

References: Millán-Leiva, A., Marín, Ó., De la Rúa, P., Muñoz, I., Tsagkarakou, A., Eversol, H., Christmon, K., VanEngelsdorp, D., & Gonzalez-Cabrera, J. (2021). Mutations associated with pyrethroid resistance in the honey bee parasite *Varroa destructor* evolved as a series of parallel and sequential events. Journal of Pest Science, 1–13.

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Distribution of pyrethroid and amitraz resistance in Varroa destructor mites from honey bee (Apis mellifera) colonies in Portugal

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Honey bees (Apis mellifera) are widely used for pollination and honey production worldwide. However, they face a serious threat from the ectoparasitic mite Varroa destructor. This parasite causes varroosis and transmits multiple viruses, such as the Deformed wing virus (DWVV), compromising the health and survival of colonies. While there are many acaricides commercially available to treat colonies against varroa, the recurrent use of certain molecules is leading to the development of varroa resistance. In Portugal, two classes of synthetic compounds are currently used: pyrethroids (fluvalinate and flumethrin) and formamidines (amitraz). However, the excessive and repeated use of acaricides has led to the development of resistance in mite populations. Pyrethroid resistance is linked to mutations in the Voltage-Gated Sodium Channel (VGSC) gene, with amino acid substitutions, at position 925 and 918. Amitraz resistance is associated with mutations in the Octopamine-like β-adrenergic receptor (Octβ2R). The amino acid changes include asparagine (N) to serine (S) at position 87 (N87S), observed in France; tyrosine (Y) to histidine (H) at position 215 (Y215H), in the USA; phenylalanine (F) to leucine (L) at position 290 (F290L), in Spain; and tyrosine (Y) to phenylalanine (F) at position 337 (Y337F), in Turkey. The distribution of resistance alleles has not yet been explored in Portugal. To fill this data gap, Varroa mites from honey bee colonies across various regions of the country were analyzed through DNA extraction, PCR with specific primers, and Sanger sequencing. The results confirmed the of widespread distribution of resistance alleles to pyrethroids at positions 925 and 918, like those observed in Spain. In contrast, preliminary results did not detect any amitraz resistance alleles, although the small sample size warrants caution in interpretation. This study represents an important step in understanding V. destructor resistance to chemical treatments in Portugal, providing essential data for monitoring acaricide resistance and supporting the development of more effective management strategies to help beekeepers better protect their colonies.
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