Varroa destructor resistance to acaricides :

Current situation in France, impact on treatment efficacy and outlook

Benjamin POIROT, PhD
APINOV, Research center (FR)

APIMONDIA 08/09/2023

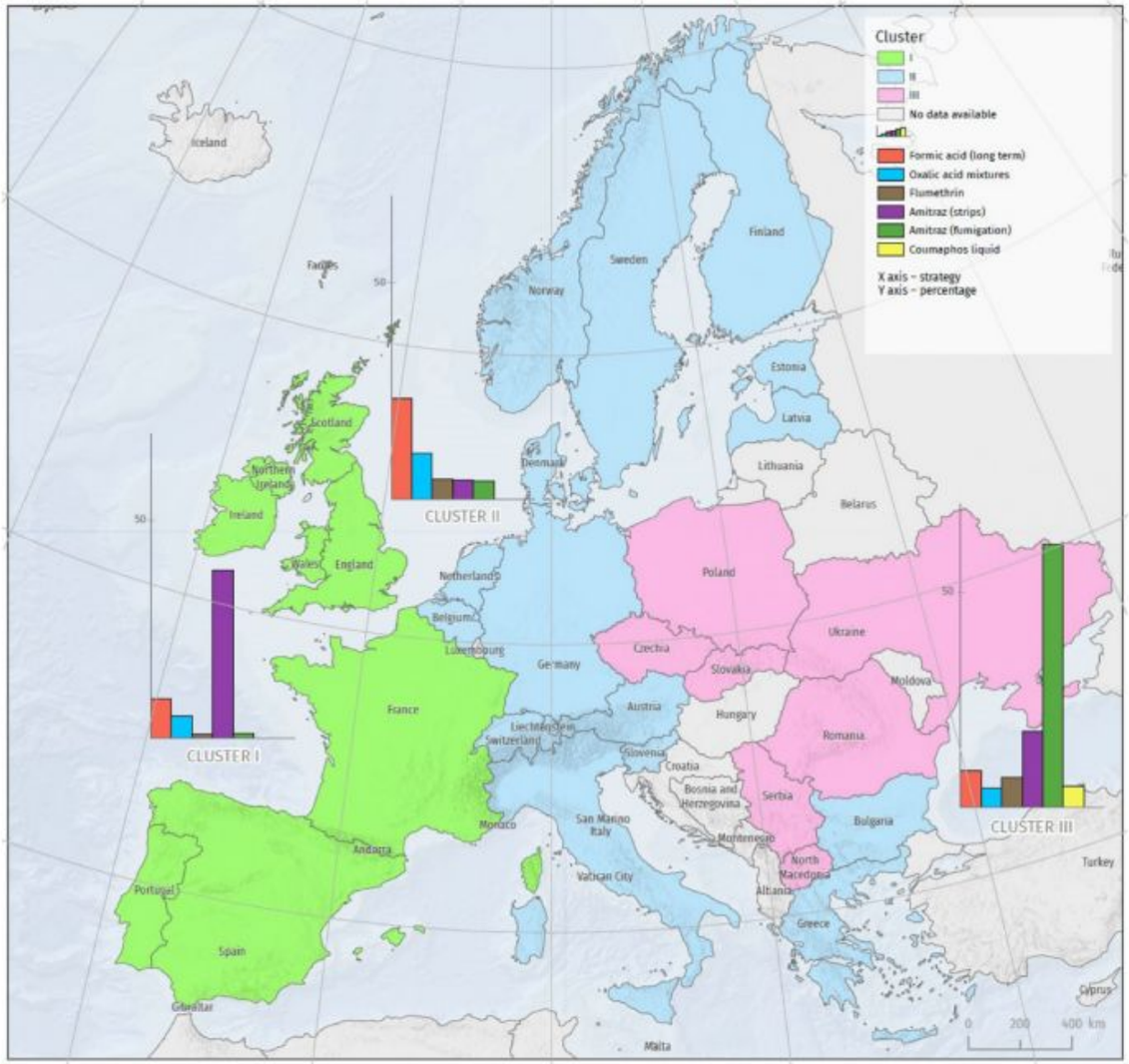
Spatial representation of the three *Varroa* control clusters identified in Europe.

-

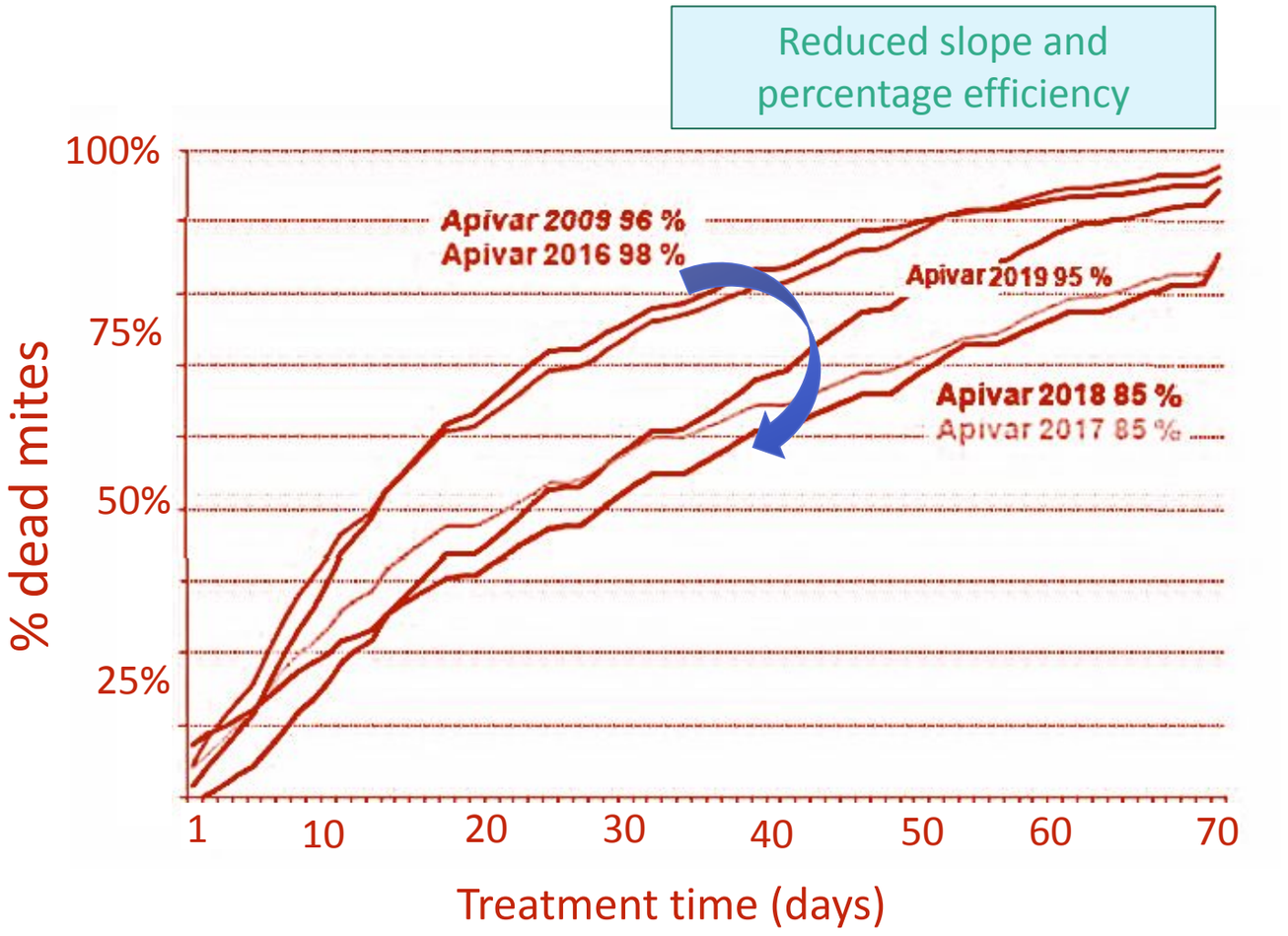
62% of colonies in Europe are treated with amitraz

Cluster 1 (Green) : **amitraz strips is the first method of treatment**

Brodschneider et al. 2022 (Journal of pest Sci)



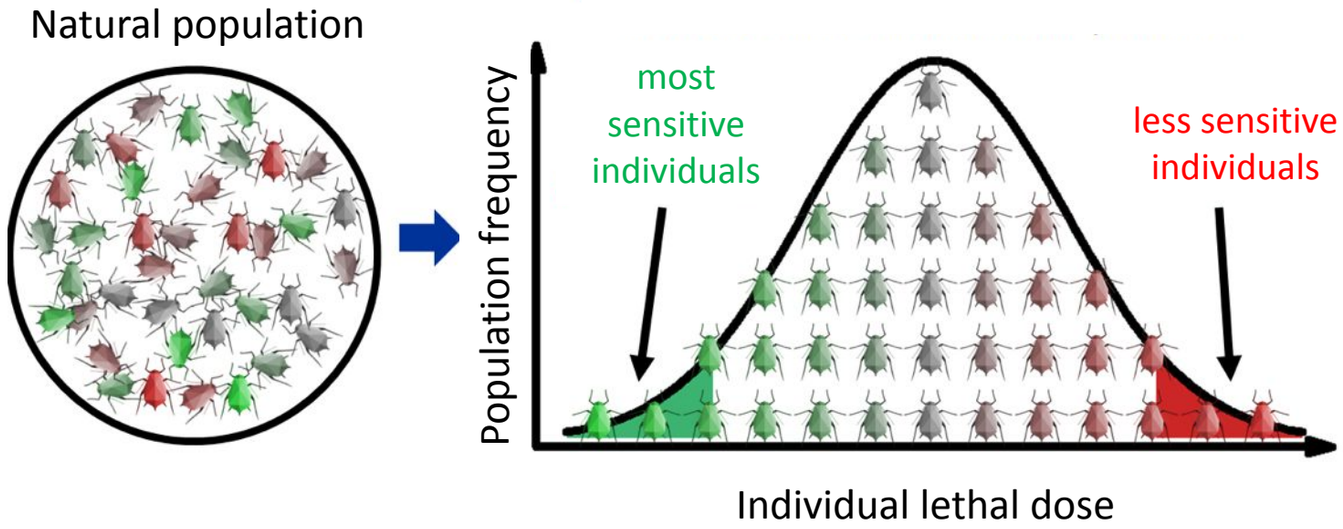
The beginning of the story…



La santé de l'abeille 2018

(Vannosthuyse et al. 2018)

Resistance types



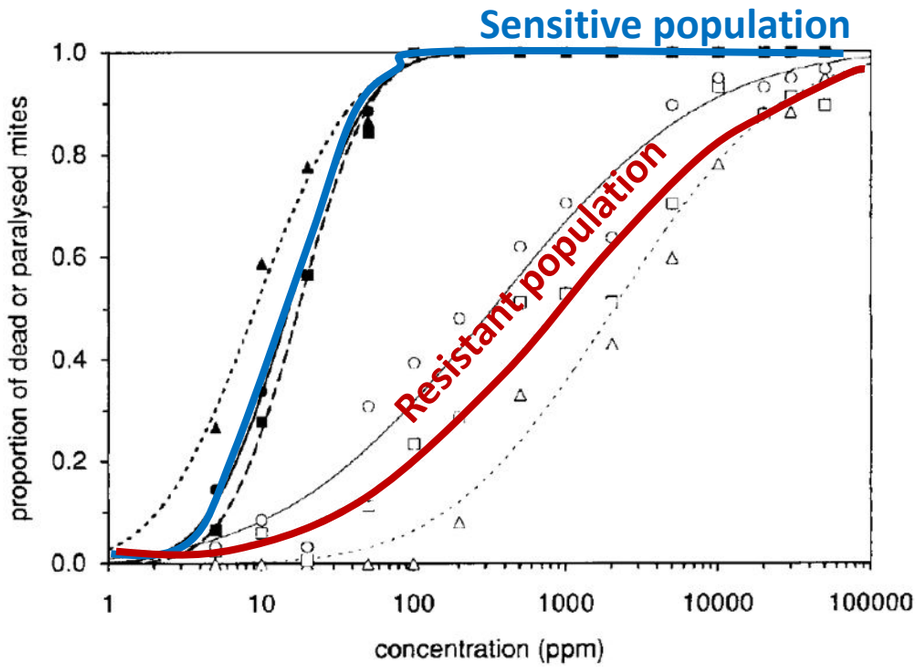
- 4 types of resistance
  - Behavioral
  - Physiological
  - Metabolic
  - Target mutation

- Known Varroa resistances for fluvaliante : Metabolic & Target mutation

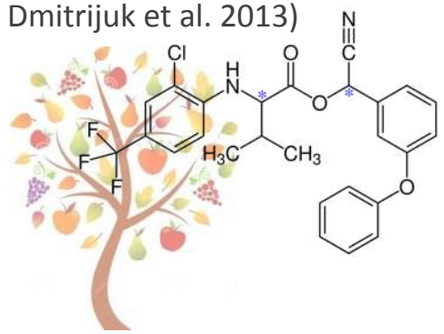
1. Context

Tau-fluvalinate resistance

- **Phenotypic test** (Milani. 1995)
  - Mortality rate / Concentration
  - **Sensitive** vs. **resistant** population



- **Enzyme test**
  - Detoxifying enzyme activity
  - Esterases (Sammataro et al. 2005; Dmitrijuk et al. 2013)

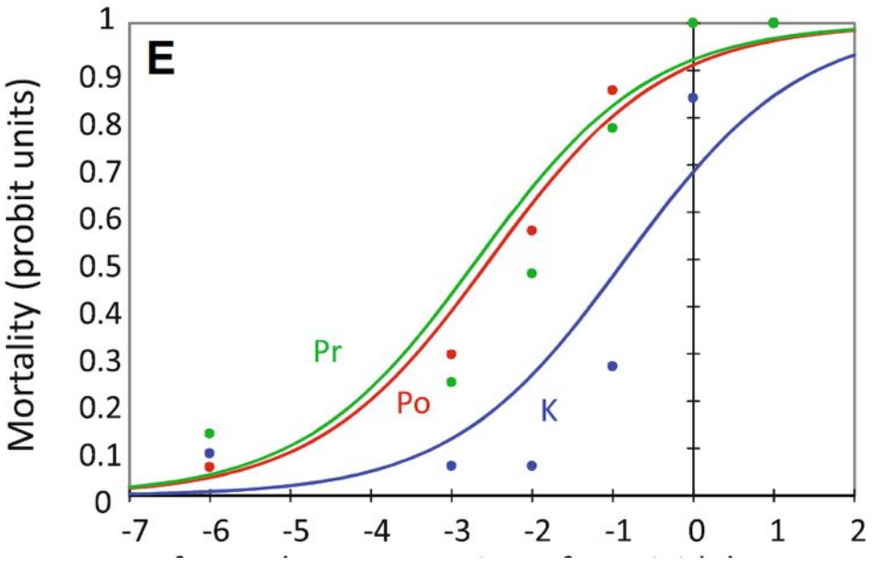


- **Molecular testing**
  - Homozygous sensitive profile (SS), Homozygous resistant profile (RR) (Gonzalez et al. 2013; 2016)
  - Several possible mutations (Gonzalez et al. 2018)
  - Europe: L925V majority

Amitraz resistance

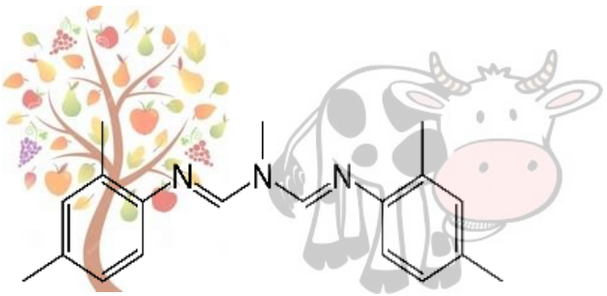
- Phenotypic test
  - Mortality rate / Concentration

Kamler et al. 2016



- Enzyme test (*Tetranychus sp*)
  - Increase in esterases

Van Leeuwen et al. 2005



- Molecular testing
  - Octopamine receptor mutation (*Boophilus* sp)

Chen et al. 2007; Corley et al. 2013

Method

1. Petri dish contamination

- Preparation of hexane-based solutions
- Several concentrations
- Control: Hexane

Sending of infested brood by beekeepers, veterinarians, Association of beekeerpers

2. Varroa collection (brood)

- Collection of mature foundresses
- 4 replicates of 15 varroa = 60 varroa / concentration

3. Contacting varroa mites (1h)

- Oven: 30±1°C, HM 60±10%.

4. Transfer of varroa mites to non-contaminated boxes

5. Mortality observation (24h)

- Oven: 30±1°C, HM 60±10%.

Almecija et al. 2020 Inventory of Varroa destructor susceptibility to amitraz and taufluvalinate in France (Exp. and Applied Acaralogy)

Method

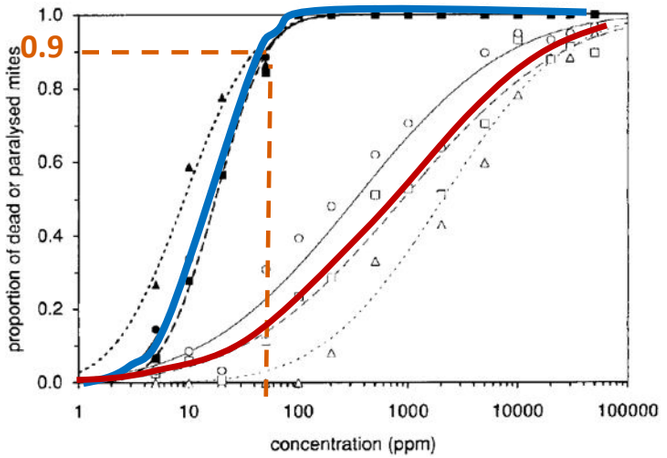
Almecija et al. 2020 (Exp. and Applied Acaralogy)

- Comparison of population mortality with sensitive reference populations

- Choosing a discriminating concentration **LC90**

LC90 [Amitraze]=0.4 µg/mL

LC90 [Tau-fluvalinate]=20 µg/mL



- Description of 3 sensitivity classes: Strong Resistance, Moderate Resistance, Sensitive

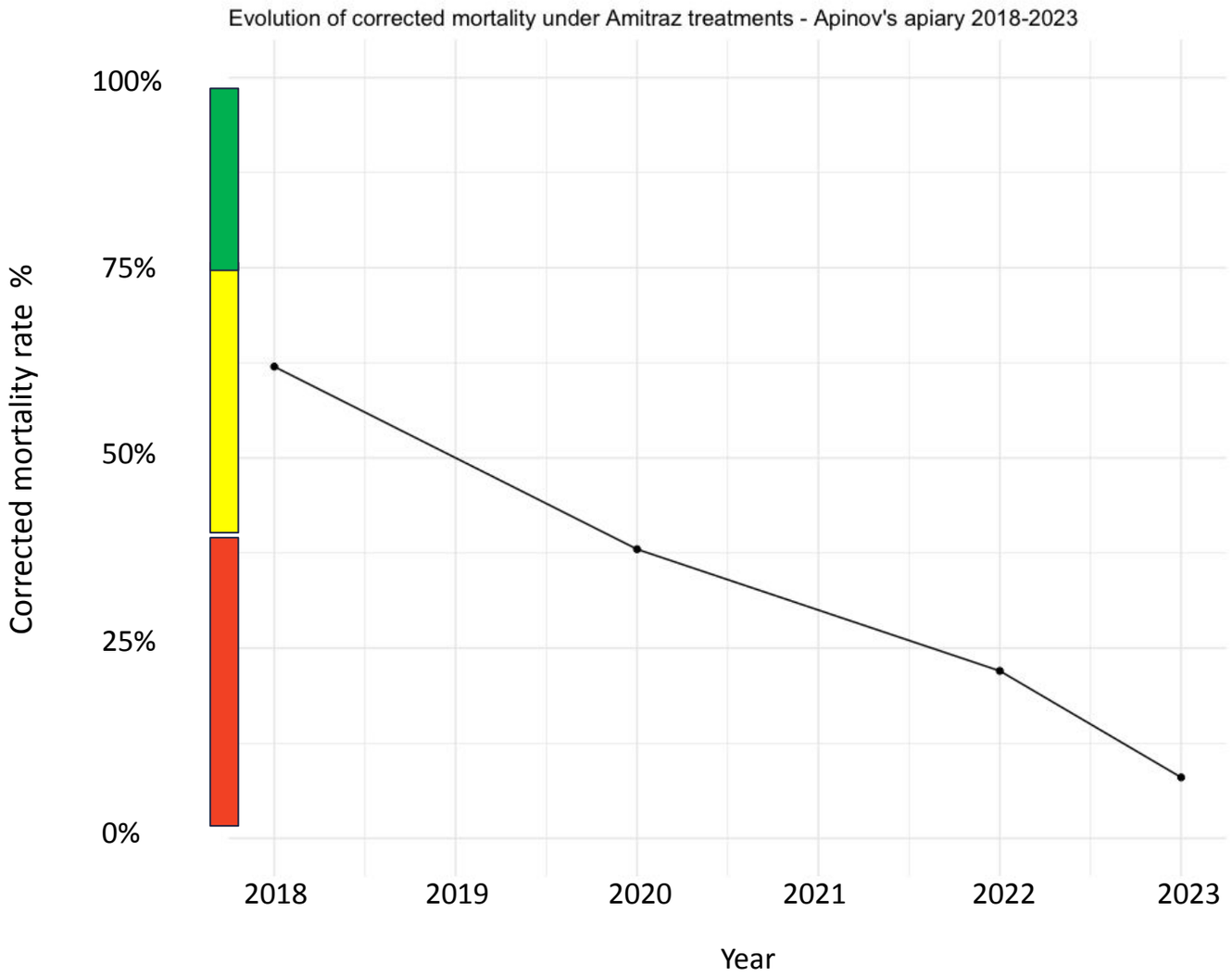
Mortality <40

Mortality 40-75%.

Mortality >75%

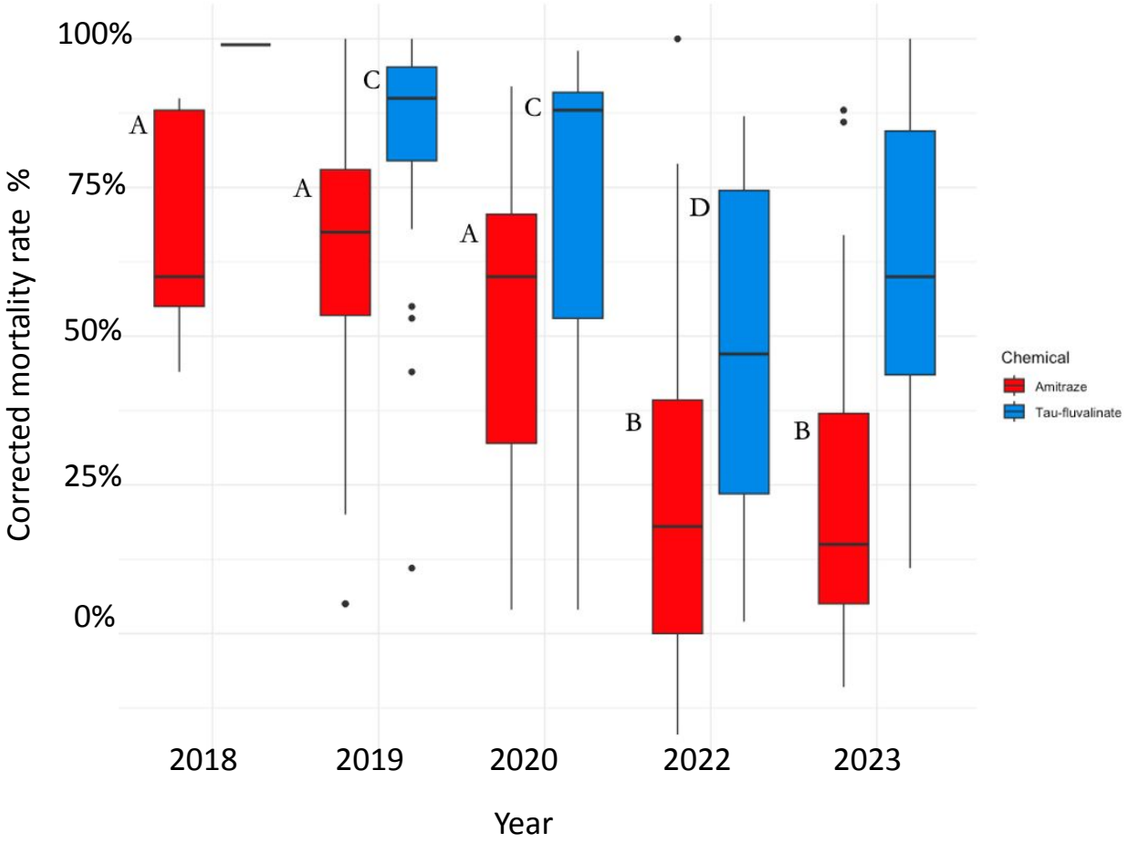
Evolution of amitraz resistance in Apinov’s apiary

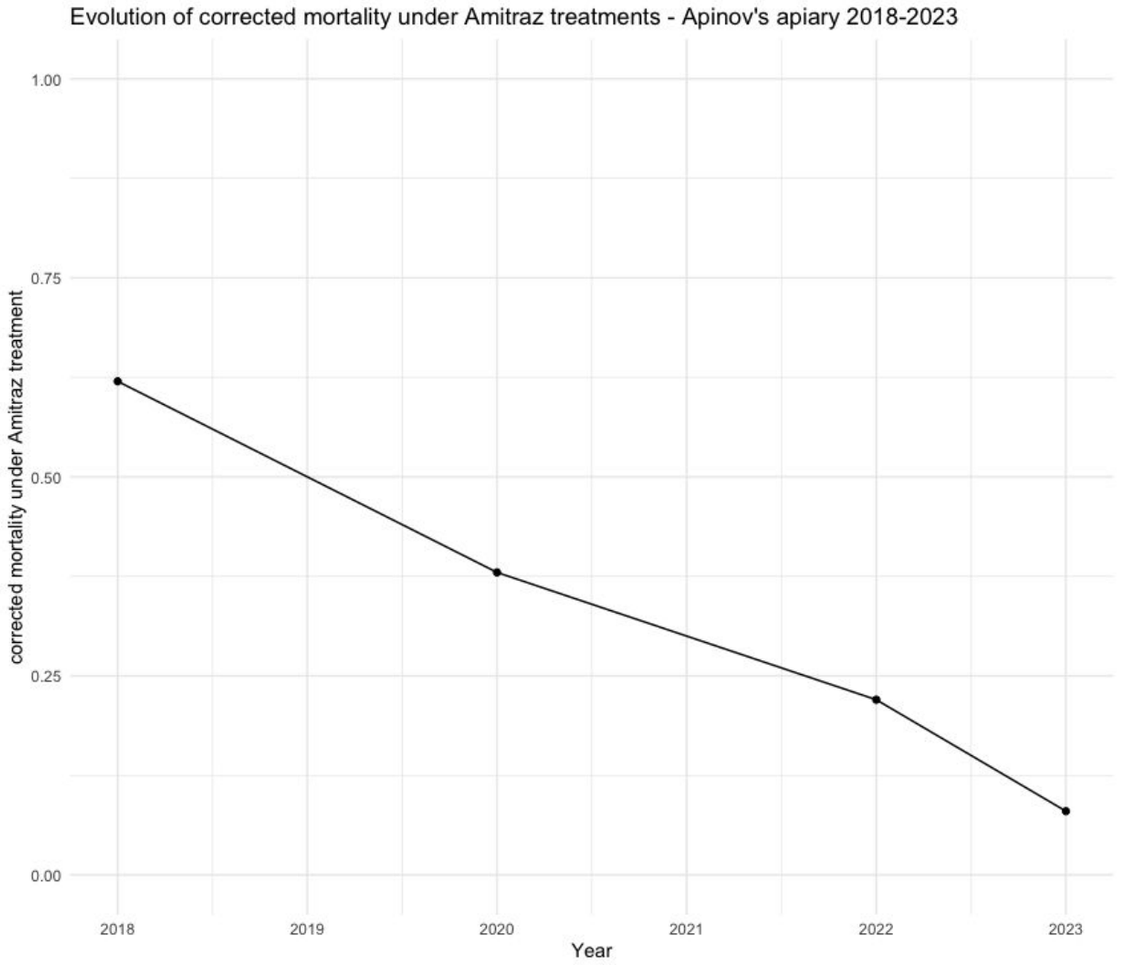
Not published data



5 y of phenotypic test results (2018-2023) in France

Not published data

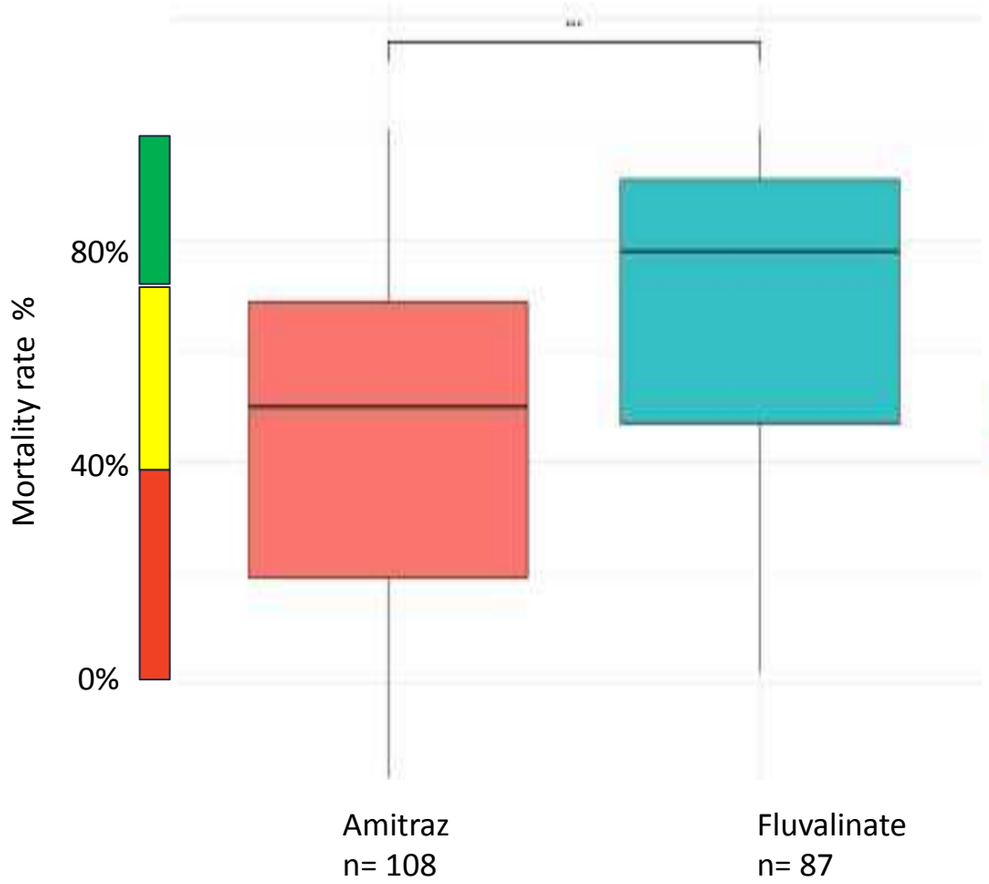




Evolution of Amitraz (n=108) and Tau fluvalinate (n=87) sensitivity through year. Different letters mean significantly different groups (Dunn Test post-hoc, $p_{val} < 0.05$)

5 y of phenotypic test results (2018-2023) in France

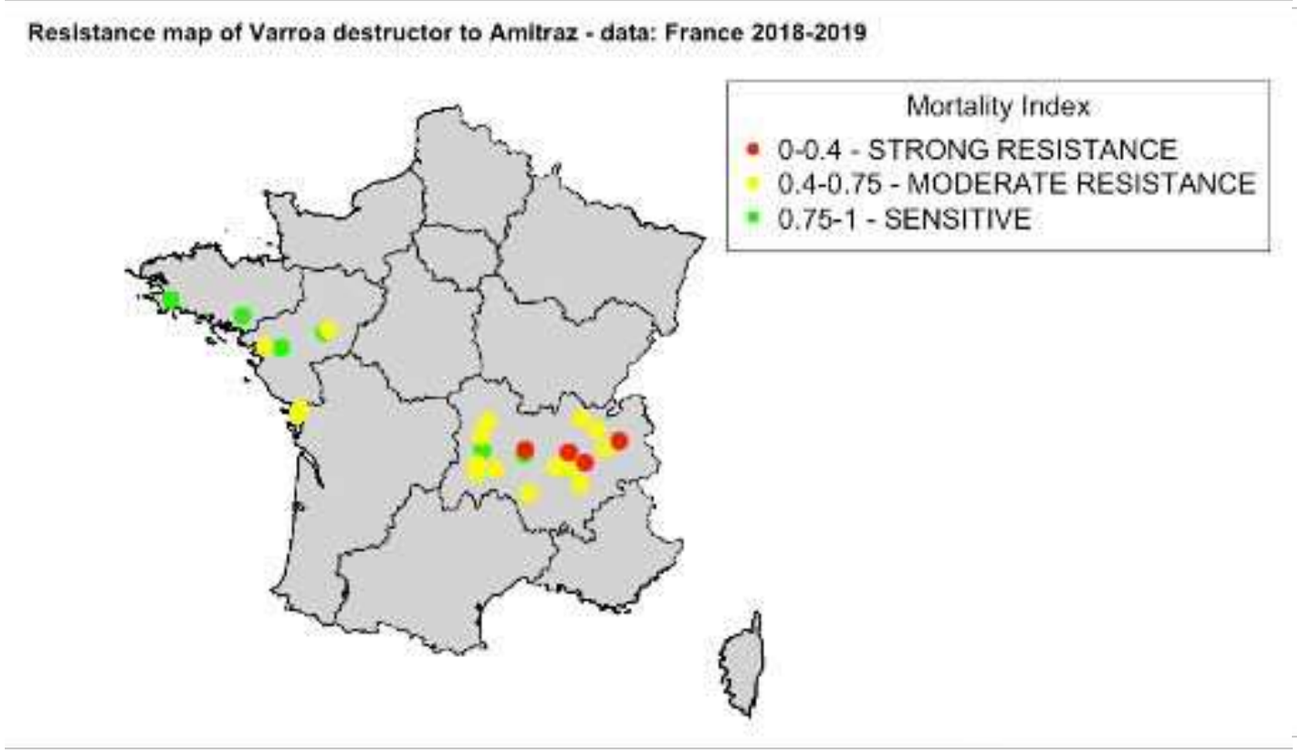
Not published data



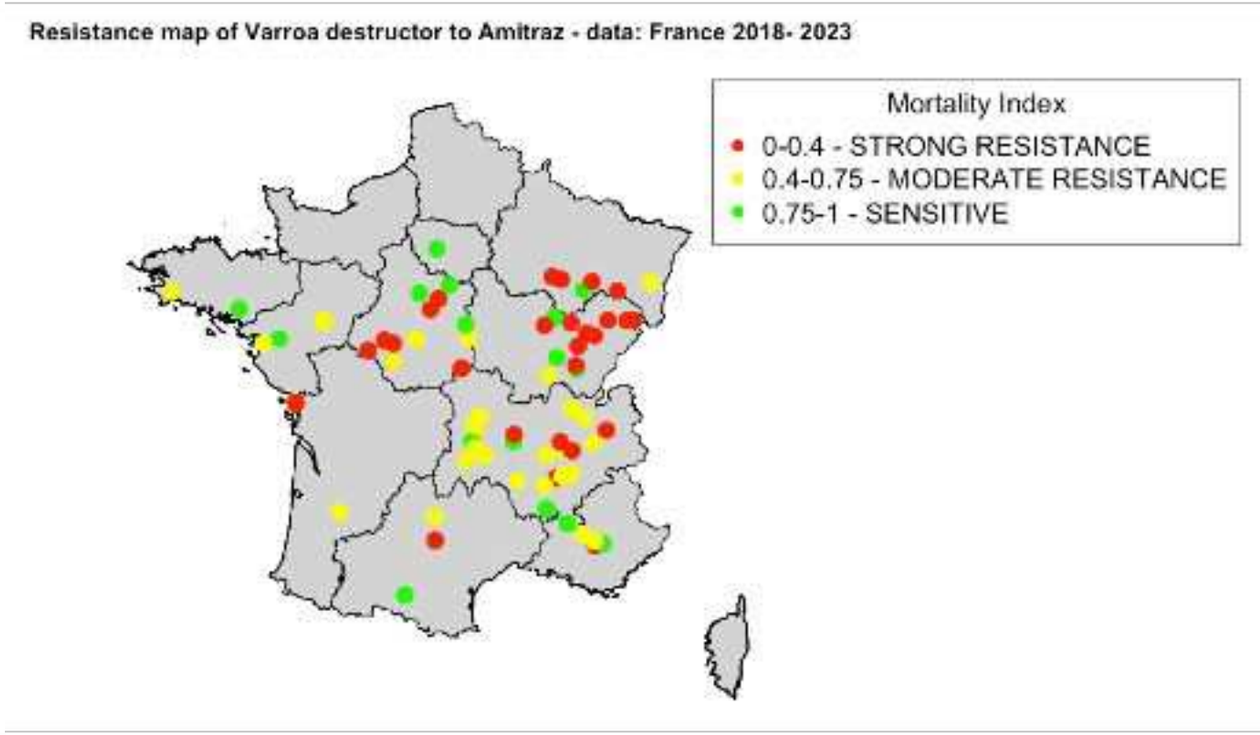
corrected mortality plots for both acaricides (data: France 2018-2023, Apinov). Student test, $p_{val} = 1.9217.10^{-5}$

Amitraz

- High heterogeneity



Almecija et al. 2020 (Exp. and Applied Acaralogy)

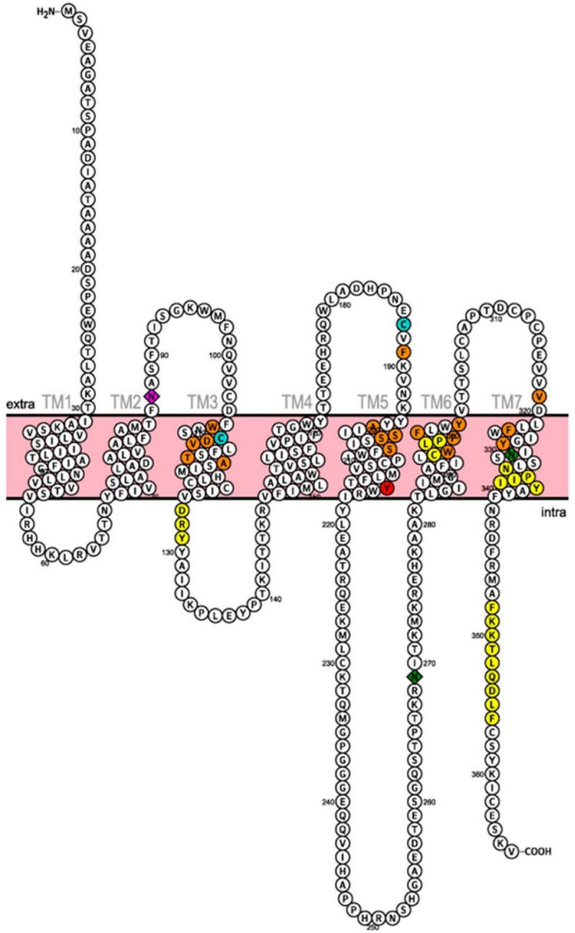


Not published data - n= 109

Amitraz: Target mutation

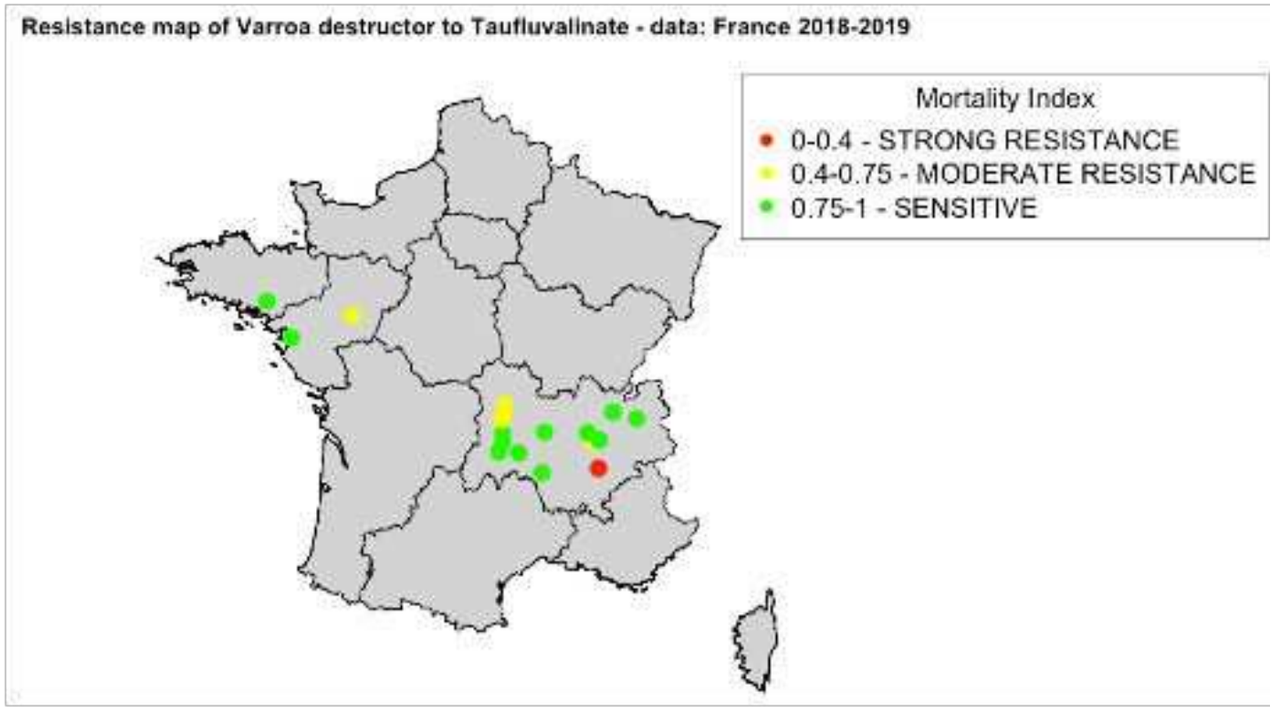
Hernandez-Rodriguez et al, 2021

« Resistance to amitraz in the parasitic honey bee mite Varroa destructor is associated with **mutations in the β-adrenergic-like octopamine receptor** »

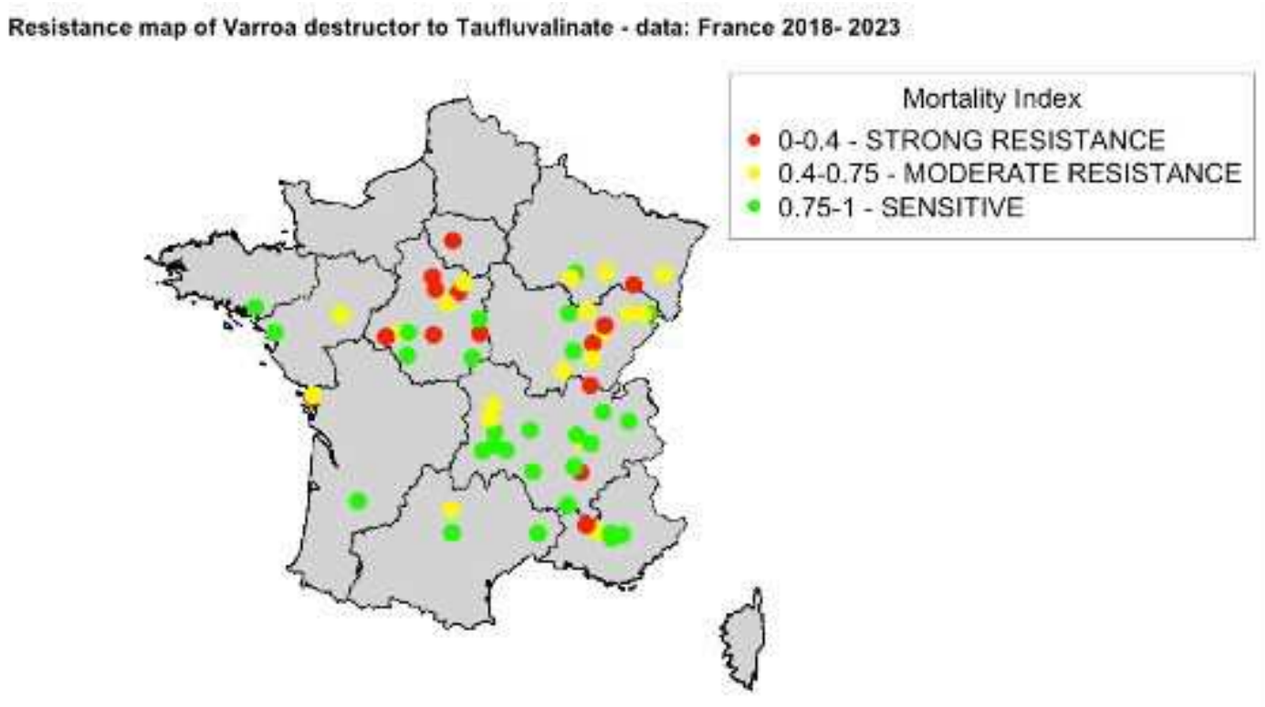


Fluvalinate

- High heterogeneity



Almecija et al. 2020 (Exp. and Applied Acaralogy)



Not published data

Tau fluvalinate :
Reversion time & target mutation

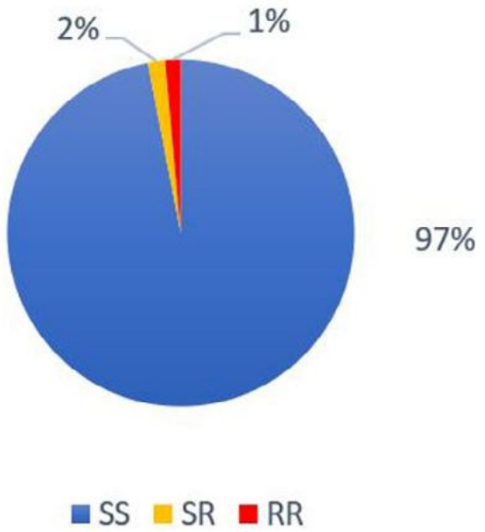


Figure 4. Distribution of the genotypes for L925V mutation for colonies not treated with Apistan® in the last 2 years (Organic and Amitraz classes). The SS corresponds to homozygous susceptible profile. The RR correspond to the homozygous resistant profile and the RS are heterozygous mites.

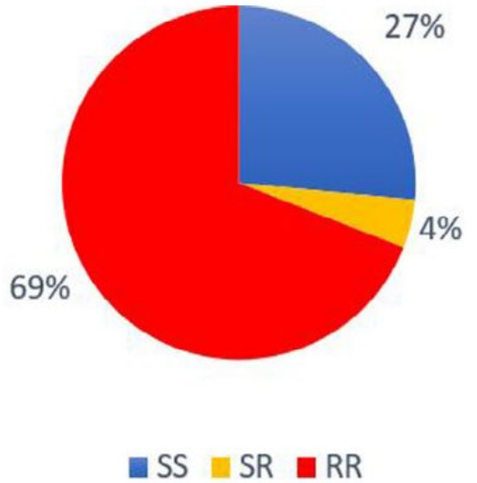


Figure 5. Distribution of the genotypes for L925V mutation for colonies treated with Apistan® in the last 2 years (Mixte class). The SS corresponds to homozygous susceptible profile. The RR correspond to the homozygous resistant profile and the RS are heterozygous mites.

Almecija et al. Varroa destructor resistance to tau-fluvalinate: relationship between in vitro phenotypic test and VGSC L925V mutation. 2022 (Pest Manag Sci)

Tau fluvalinate : Reversion time & target mutation

Almecija et al. 2022 (Pest Manag Sci)

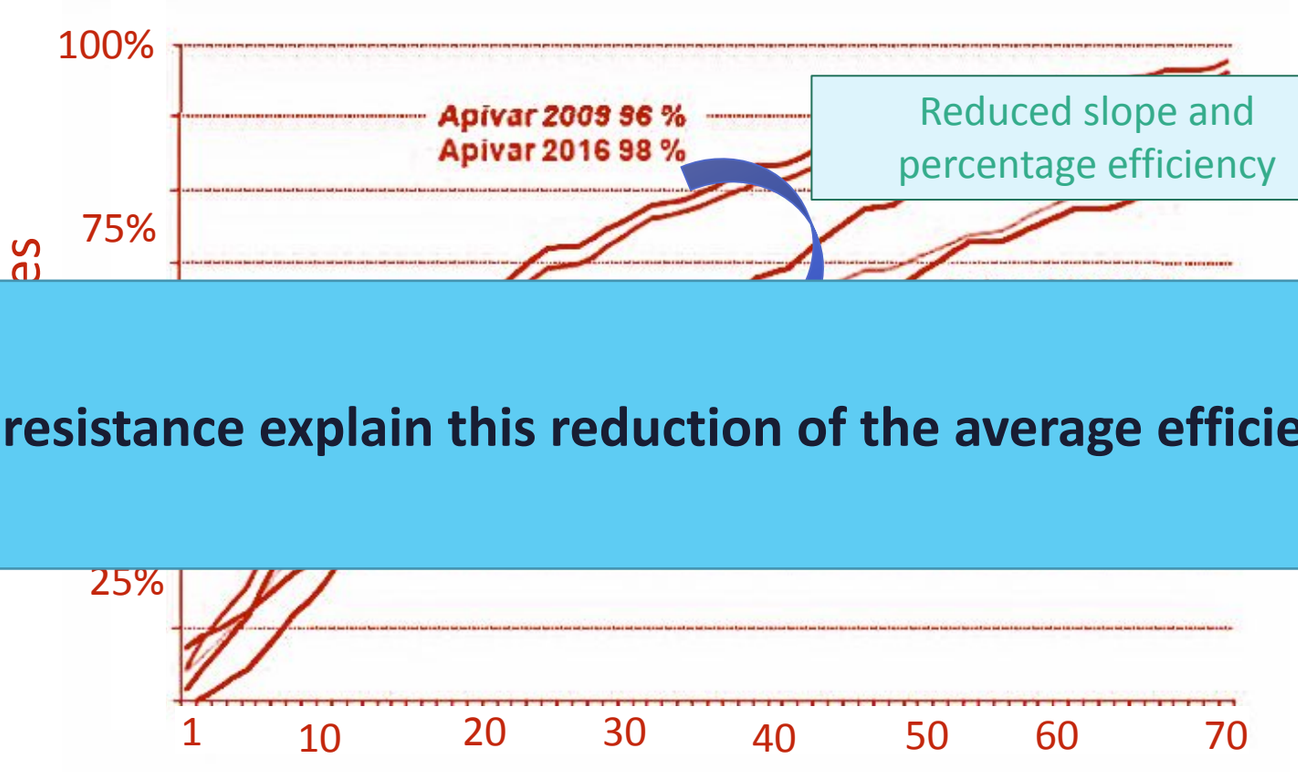
Table 3. Susceptible Detection Reliability and Resistant Detection Reliability of the molecular test for the evaluation of resistance to *tau*-fluvalinate. The TFDE and TFDC populations were excluded from the analysis as the dead and surviving mites were not separate after the phenotypic test

	Number of *Varroa* with the susceptible phenotype	Number of *Varroa* with the resistant phenotype	Total
Number of *Varroa* with the susceptible genotype (SS and SR)	368	73	441
Number of *Varroa* with the resistant genotype (RR)	12	122	134
Total	380	195	575
Susceptible Detection Reliability[a]	0.97	—	
Resistant detection Reliability[b]	—	0.63	
Confidence interval (95%)	0.95–0.98	0.56–0.69	

[a] The Susceptible Detection Reliability of the molecular test is defined by the ability to detect susceptible genotyping population among the *Varroa* population susceptible to *tau*-fluvalinate *in vitro* exposure.
[b] The Resistant Detection Reliability of the molecular test is defined by the ability to detect resistant genotyping population among the *Varroa* population resistant to *tau*-fluvalinate *in vitro* exposure.

Almecija and all, 2021 ***Pest Manag Sci***

Resistance & Efficiency



Reduced slope and percentage efficiency

Can resistance explain this reduction of the average efficiency?

3. Modelization

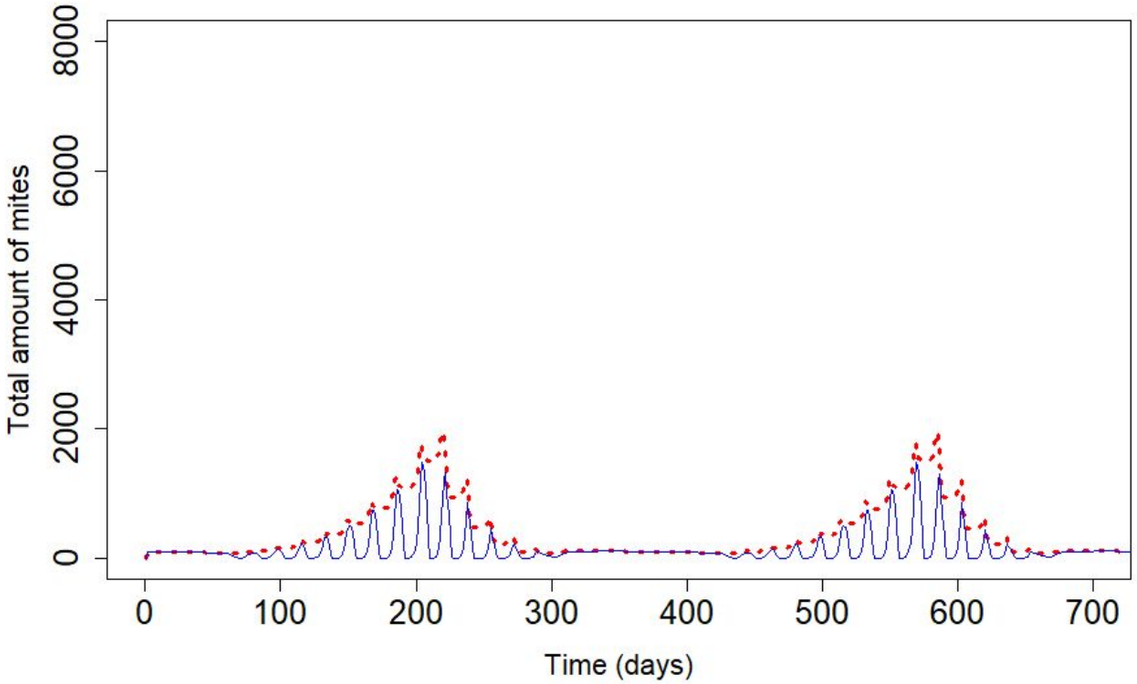
Results with APIVAR® treatment

Initial condition: pT= August 8, dT=10 weeks, Pi= 100 varroa mites

Varroa mites on bees

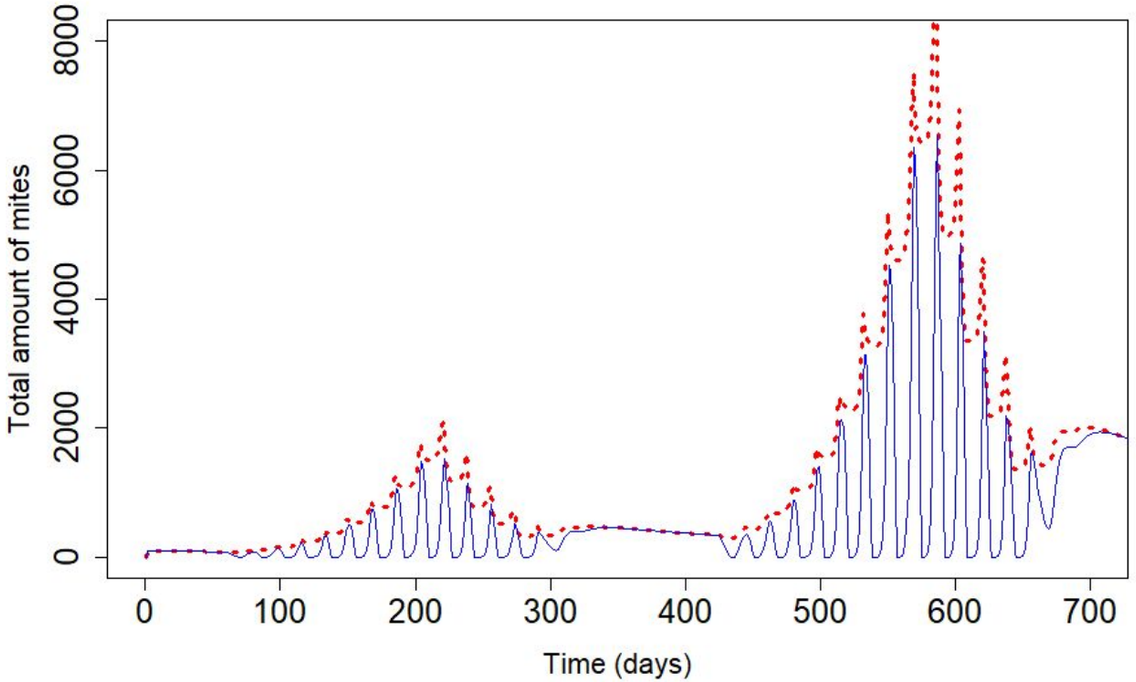
Total mites

Population stabilization



Model efficiency: 98.7

Population growth



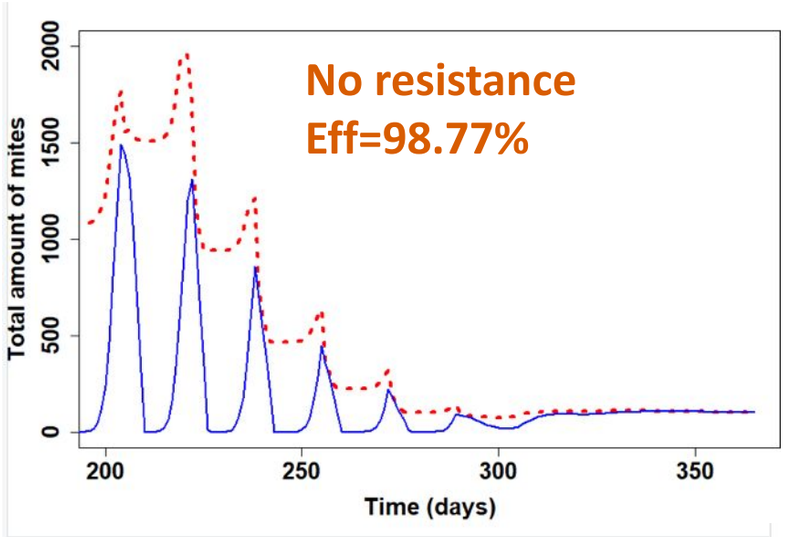
Model efficiency: 95%.

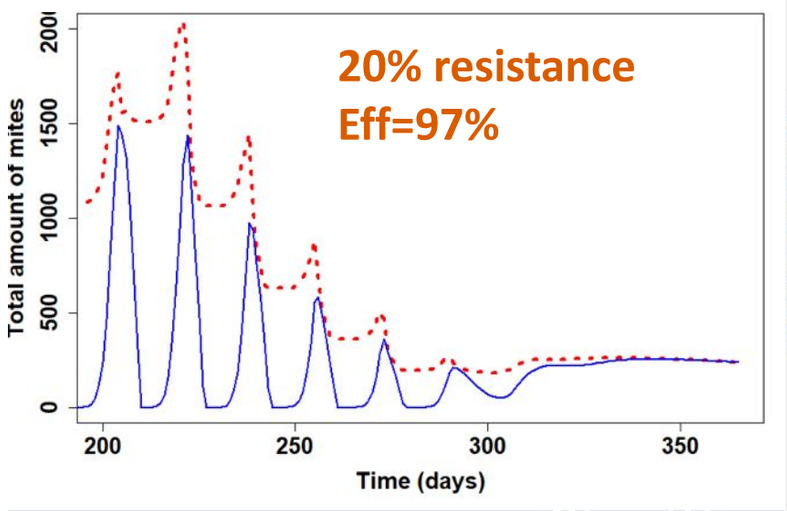
Almecija and all, ***Modelling the impact of Apivar treatment on a Varroa mite population and the influence of resistance.*** *2021* ***Pest Manag Sci***

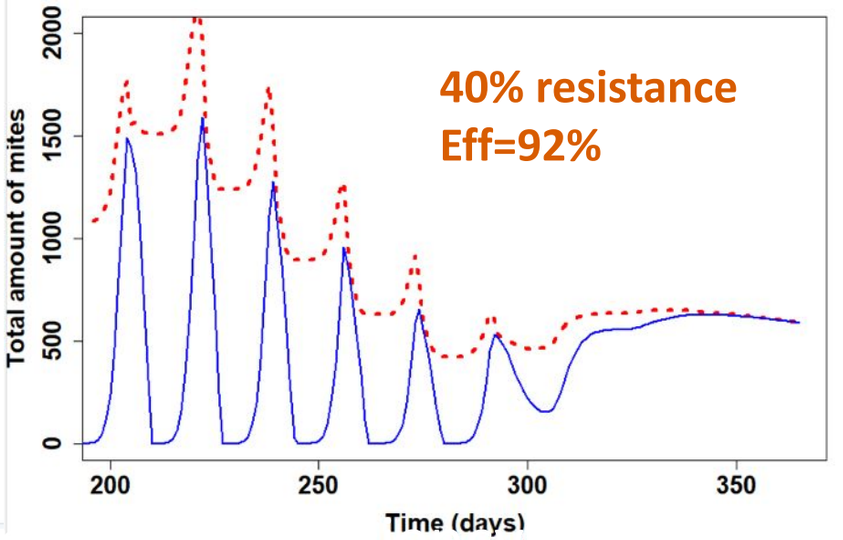
3. Modelization

Almecija and all, 2021 ***Pest Manag Sci***

Influence of a resistance on efficiency

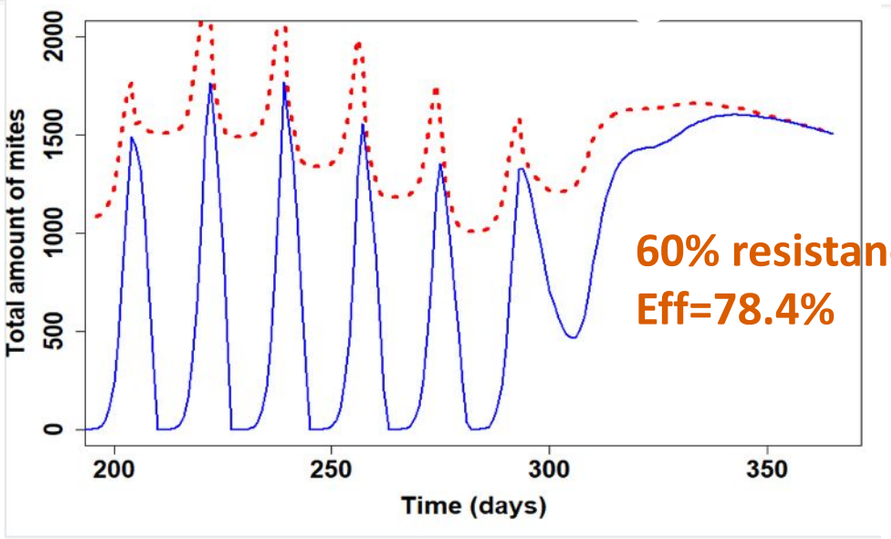






Phoretic Varroa Mites

Total mites



Comparable to field observations

Average resistance 60% in lab

=

Average efficiency 81% in field

4. Discussion

→ **Amitraz resistance is confirmed** in France and increases to a worrying level
→ **Varroas** seem to be **more sensitive to fluvalinate than amitraz** in France (Apinov's data)
→ The **reversion period** of fluvalinate is more than 2y and unknown for amitraz
→ **Integrated Pest Management** is the only way for a sustainable beekeeping :

- Reduction of selection pressure : Rotation (more than 1y)
- Reduction of treatment duration => Queen caging / Bitherapy
- Mosaic treatment (multiple active compounds on the same apiary)
- Monitoring the resistance level

Thanks !

Technical support
Association of beekeepers & veterinarians

Scientific support
Gabrielle Almecija, Alexandre ALBO, Myriam
Laurie & Elise Poisson (Apinov)
Matthieu Liberman (AgroParisTech)
Christelle Suppo (IRBI)
Veronique Duquesne (ANSES)
Joel Gonzalez (Univ Valencia)
Paulo Mielgo (VITA beehealth)
Max Watkins (VITA beehealth)

Benjamin POIROT

Find the presentation on www.apinov.com/actualités