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Amitraz is one of the most widely used miticides for controlling *Varroa destructor*, a major honey bee parasite. However, increasing reports of amitraz resistance threaten its efficacy and colony health. This review analyzes the global status of amitraz resistance in *Varroa* populations, focusing on resistance mechanisms and laboratory bioassays that predict field performance.

The prolonged and widespread use of amitraz has led to the selection of resistant mite populations, raising concerns over its continued effectiveness. Beekeepers rely heavily on amitraz, and treatment failures are becoming more frequent. Understanding resistance dynamics and identifying potential alternatives are critical for sustainable mite management. A systematic literature review was conducted following PRISMA guidelines. A total of 206 studies were screened, with 74 selected for analysis. These studies were categorized into laboratory assays (toxicological, metabolic, genetic) and field evaluations. Data from 27 laboratory studies, 42 field studies, and 12 resistance assessments were examined, focusing on resistance ratios (RR), resistance indices (RI), and mechanisms of resistance.

Amitraz resistance in *V. destructor* has been extensively documented worldwide. Resistance ratios (RR) and resistance indices (RI) show considerable variation across mite populations. However, the genetic mechanisms underlying resistance remain largely unknown. Laboratory bioassays have demonstrated variable toxicity responses influenced by factors such as mite strain, geographical location, and experimental conditions.

Amitraz resistance is an emerging challenge for *Varroa* control in beekeeping. While laboratory bioassays provide valuable insights into resistance trends, further research is needed to elucidate genetic mechanisms and improve control strategies. The findings highlight the urgent need for integrated pest management (IPM) approaches that reduce reliance on amitraz and incorporate alternative control methods.

Beekeepers should be vigilant for signs of amitraz resistance, particularly in cases of treatment failure. Regular monitoring through laboratory assays and resistance testing is recommended to assess efficacy. Implementing IPM strategies—combining pharmacological, biotechnical, and cultural practices—will be key to sustainable *Varroa* management and the long-term health of honey bee colonies.

Sensitivity and Resistance to Amitraz and Amitraz-Based Products in honey bee Parasitic Mites

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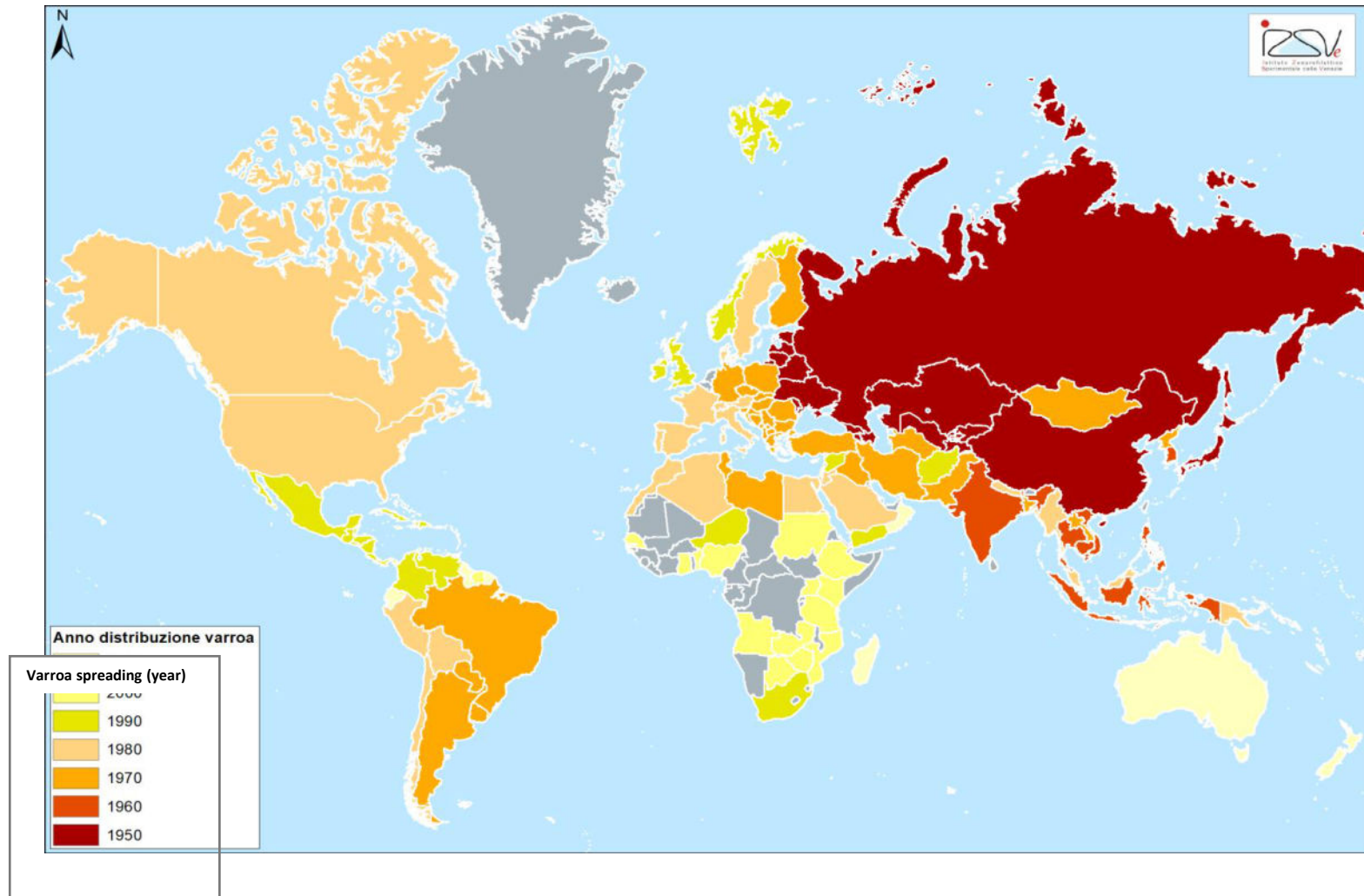
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● **Varroa:** major pathogenic agent in honey bee colonies



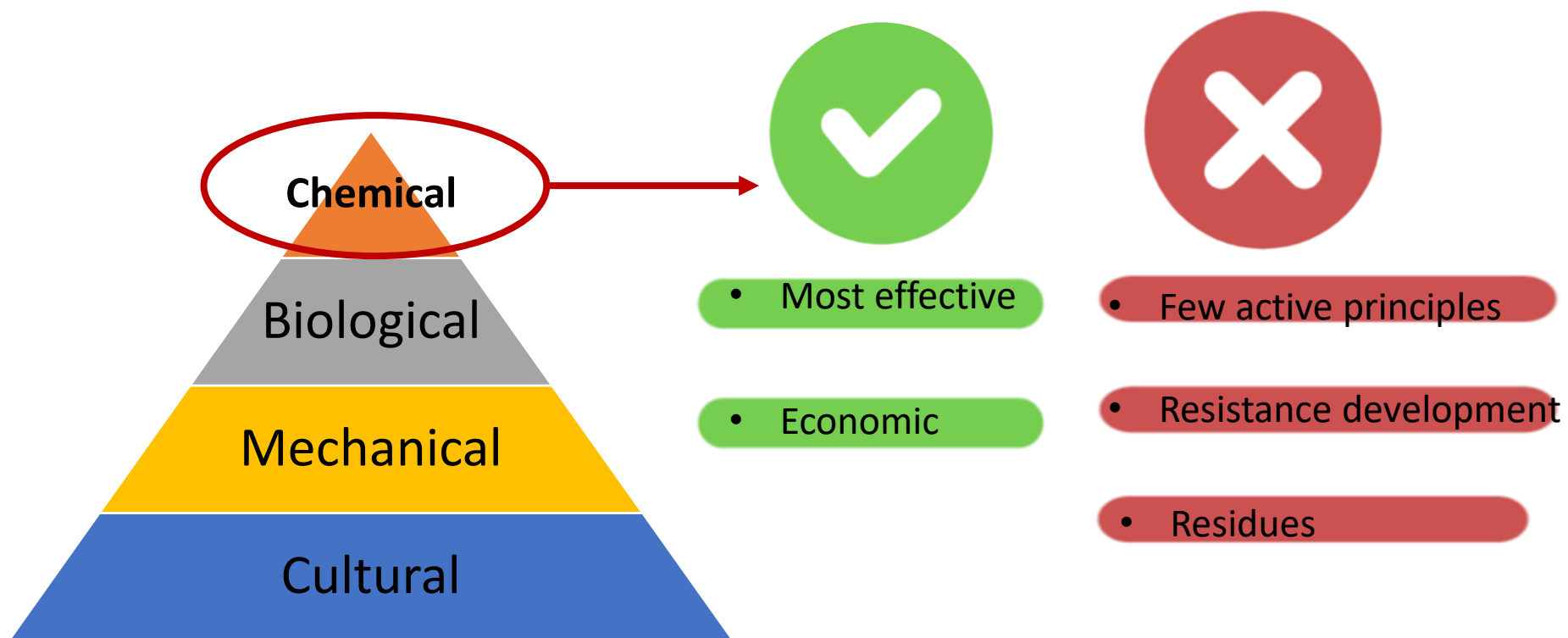
● Varroa: current worldwide distribution



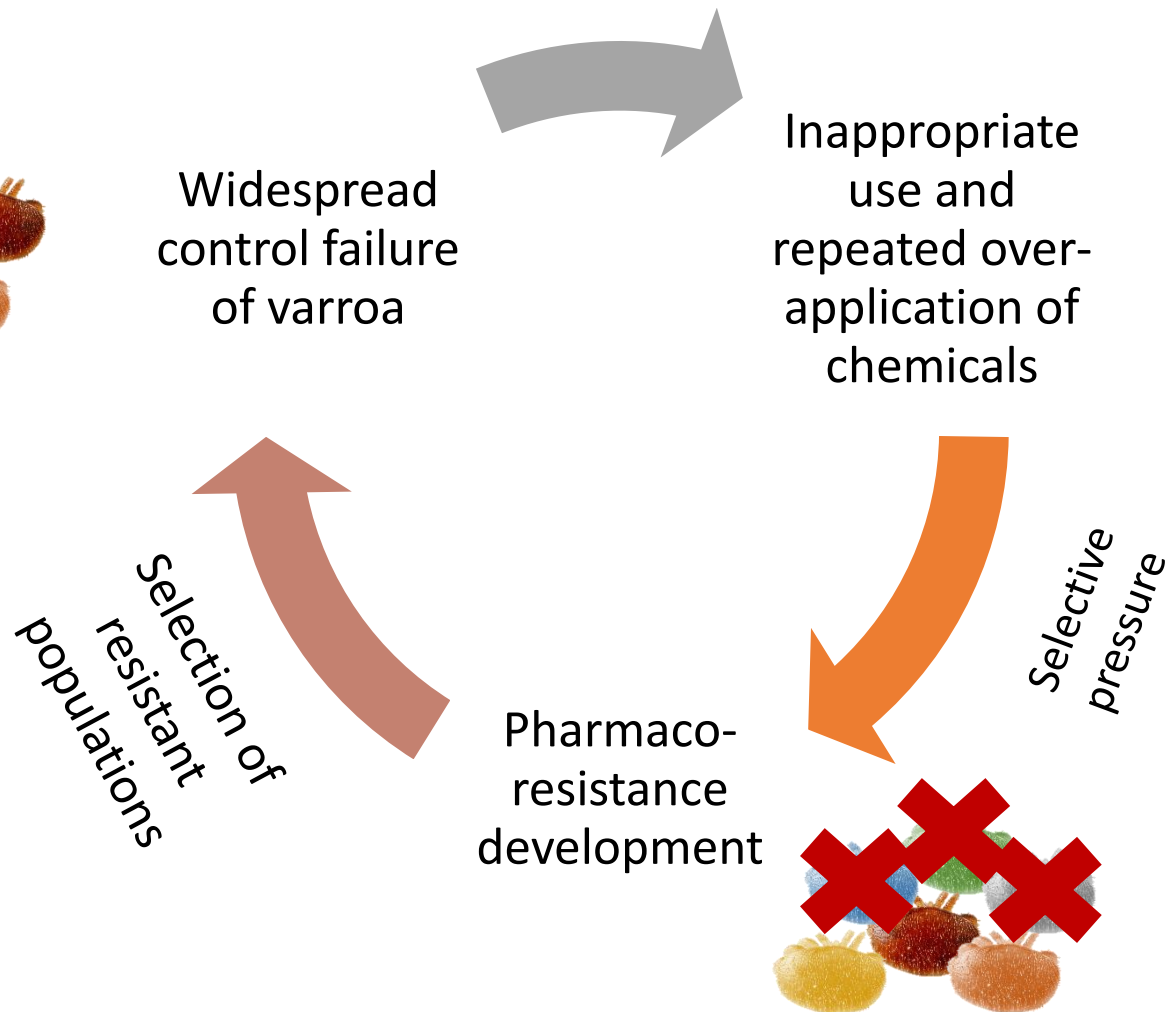
WOAH: Terrestrial animal health code (chapter 9.6).

FAO: Good Beekeeping Practices Manual and Sustainable Apiculture Guidelines (2021).

● Integrated pest management (**IPM**) strategies



● Resistance vicious cycle



● Resistance vs efficacy/sensitivity

Laboratory assays



- Toxicological assays
- Genetic assays (mutations in molecular targets)
- Metabolic assays (metabolic adaptations - changes in the expression of detoxification enzymes)



Resistance assessment

Field tests



Reduced field efficacy

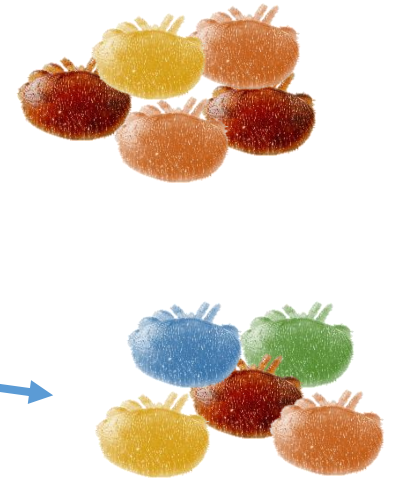


Inference on resistance status

● Toxicological assay

- Lethal concentration required to achieve 50% mortality (LC₅₀) of mite.
- Resistance Ratio (RR) or Resistance Index (RI).

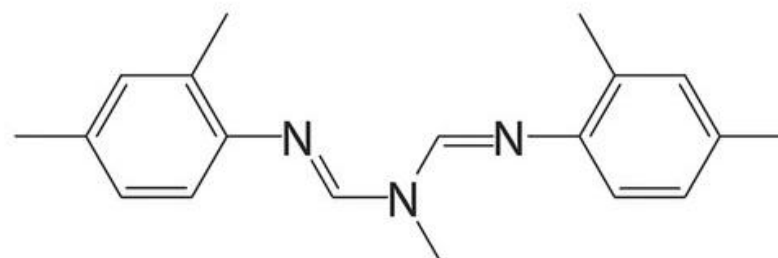
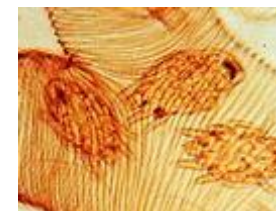
$$\text{RR or RI} = \frac{\text{LC}_{50} \text{ target mite population}}{\text{LC}_{50} \text{ susceptible mite population}}$$



- A higher RR or RI indicates a greater level of resistance in the target population.

● Aim of the study

“What is the current status of *V. destructor*, *Tropilaelaps* spp., and *A. woodi* mites’ **sensitivity** and **resistance** to amitraz and amitraz-based products”?

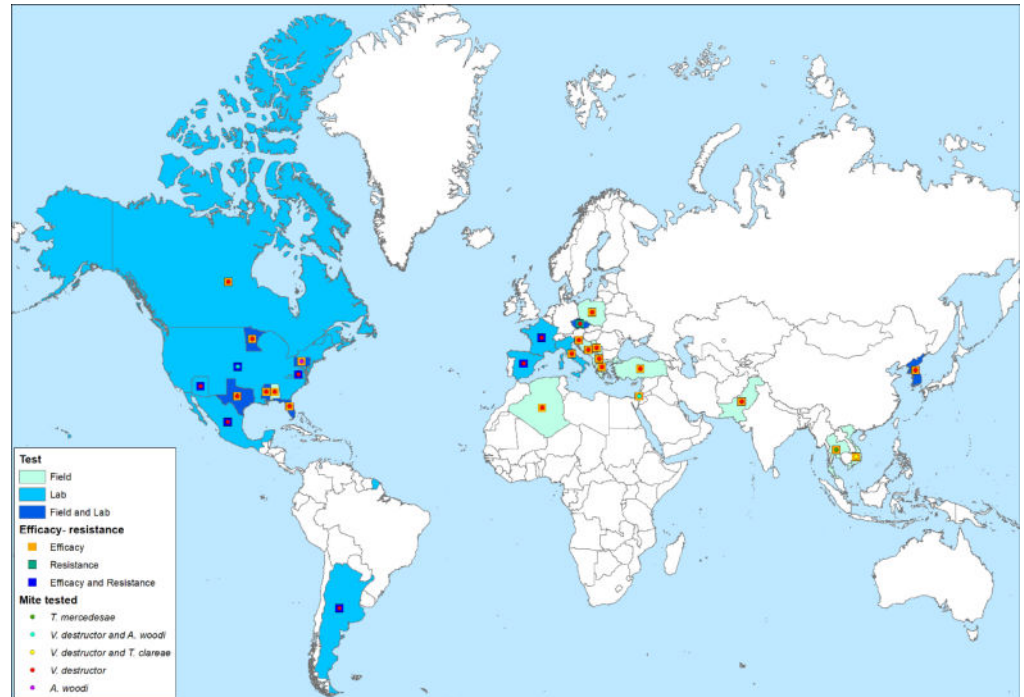


Amitraz

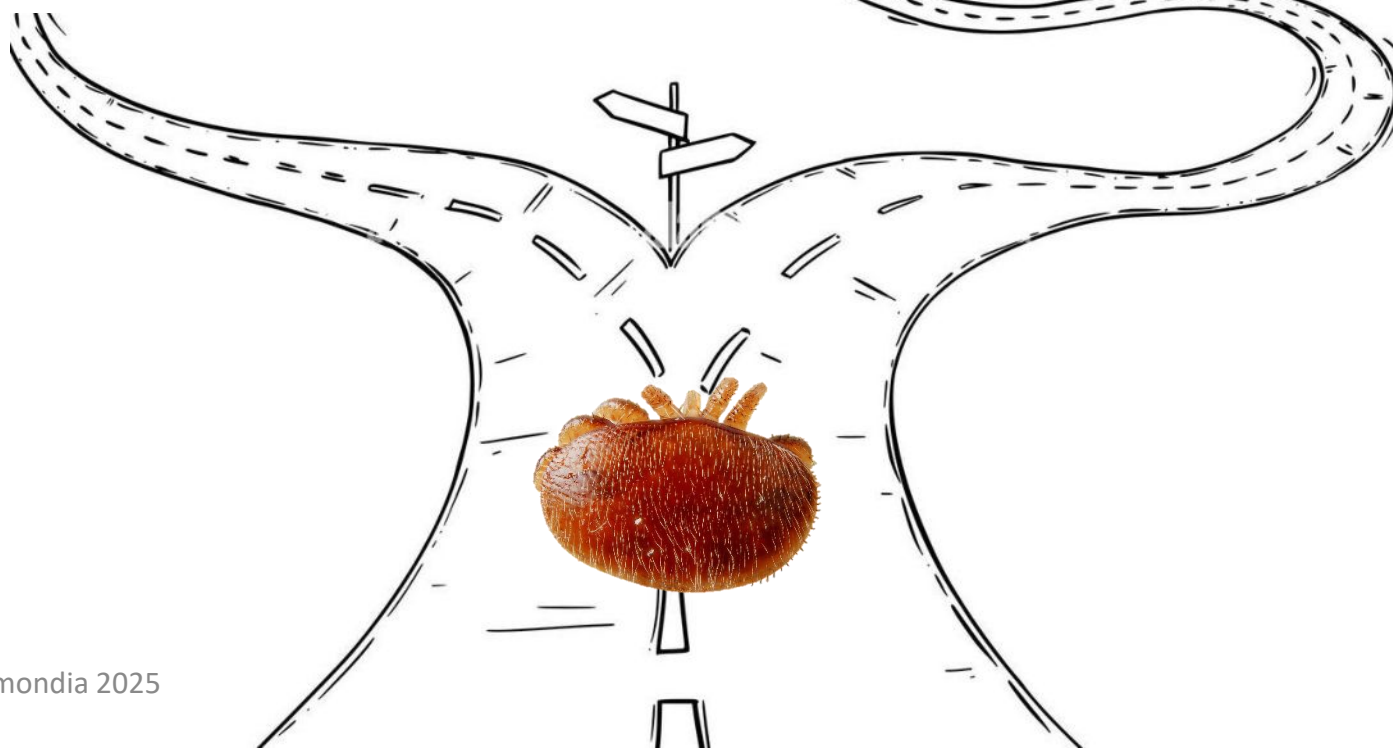
● Amitraz studies: State of art

Mite species	Laboratory studies efficacy	Field studies efficacy	Resistance assesment
<i>V. destructor</i>	27	43	12
<i>T. clarae</i> and <i>V. destructor</i>	-	1	-
<i>T. mercedae</i>	-	1	-
<i>A. woodi</i>	1	4	-

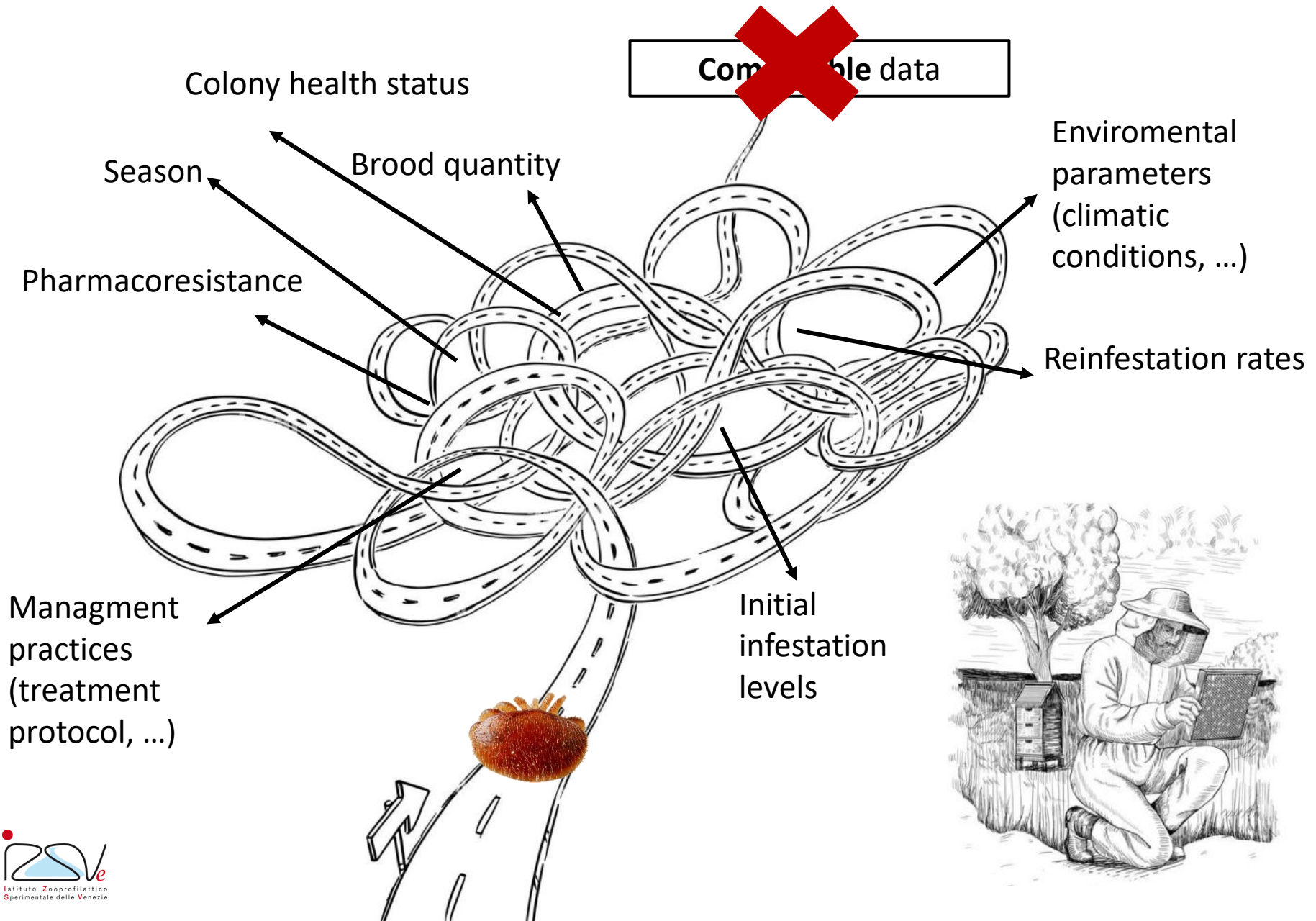
- PRISMA guidelines
- PUBMED and the Web of Science Core Collection
- Up to 3 January 2025
- English language with full-text availability
- Primary research data



● Filed and/or Laboratory tests?

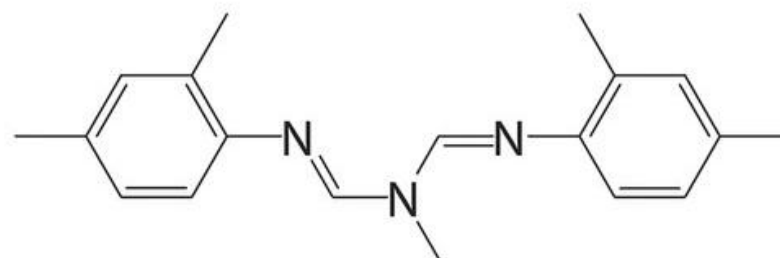
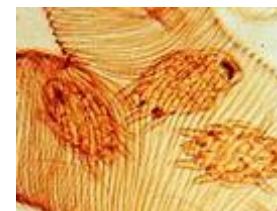


● Field tests (n=43): lack of comparability



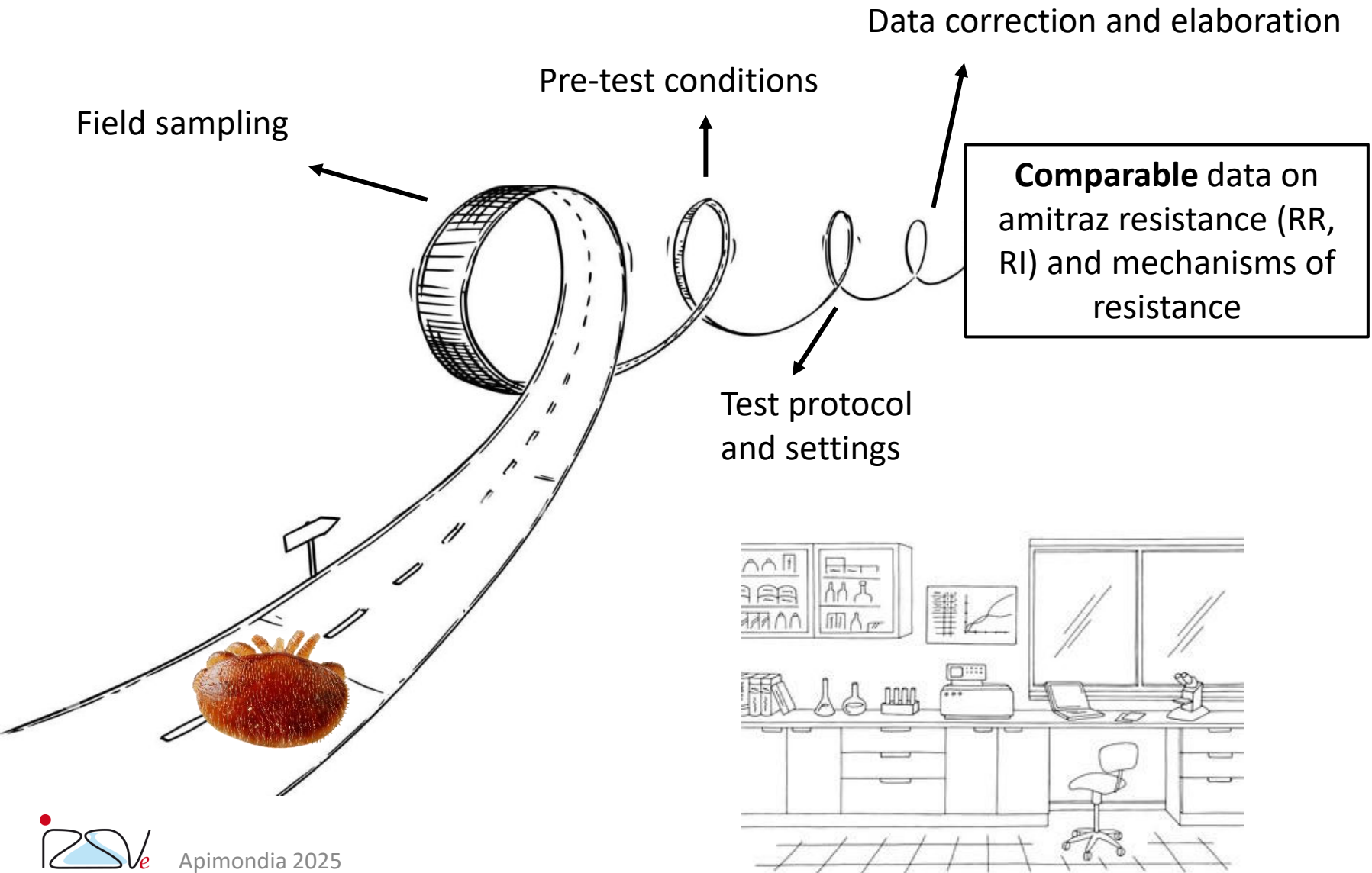
● Aim of the study

“What is the current status of *V. destructor*, *Tropilaelaps* spp., and *A. woodi* mites’ **sensitivity** and **resistance** to amitraz and amitraz-based products **based on laboratory studies**”?



Amitraz

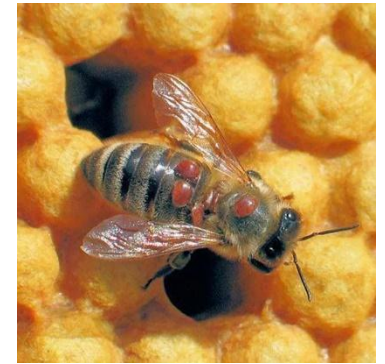
Laboratory tests: still complicated but we try to do the loop-the-loop one at a time



First loop-the-loop: from field sampling to the lab facility



- From a single colony from one apiary, a single apiary (more colonies from one apiary), or multiple apiaries (more colonies from more apiaries)
- Data from different apiaries could be aggregated or maintained as divided and then compared
- From uncapping pupae or from honey bees of mixed ages, workers, or nurse bees
- Using CO₂ or sugar shake from honey bees
- Treatment protocol used in each apiary
- Temporal collection period and geographical coordinates/details



● Second loop-the loop: Pre-test conditions



- Mite handling
- Environmental conditions
- Transport duration
- Feed
- Mite selection criteria (excluding mites that appeared freshly molted, weak, or deformed)



● **Third loop-the-loop: Test protocol (pt.1)**



- Contact toxicity assays or topical application through microapplication
- Direct contact (Petri dishes, mason jars, apiarium, glass vials, and various plastic containers) or spraying (Potter–Burgerjon tower)
- Amitraz powder (either undiluted or mixed with solvents such as hexane, acetone, or ethanol) or Veterinary medicinal products (Apitraz[®], Apivar[®], Taktic[®], Mitaban[®])
- Size of mite groups, the number of replicates

● **Third loop-the-loop: Test protocol (pt.2)**



- Experimental conditions, including temperature and humidity
- Exposure times (contact time between mites and the a.i.) ranged from one hour to 48 h, while incubation periods—the time between exposure and efficacy assessment—were reported in only few studies
- Either mites were tested alone or alongside larvae, pupae, or adult bees (minimally infested with Varroa, <1%), which could be introduced after either exposure or several hours later

● Fourth loop-the-loop: Data Correction - Elaboration

- Criteria for determining mite mortality
 - Amitraz targets the mites' octopamine receptors, disrupting synaptic transmission and causing paralysis). This paralysis is sub-lethal, with death occurring indirectly due to starvation resulting from the inability to move or feed.
 - Since Varroa mites are obligate parasites of bees and cannot survive for long after detaching from their host, their time of detachment can be reasonably approximated as their time of death
- Correction for natural mortality



Starved or paralysed?
That is the question...

● Sensitivity to amitraz and amitraz-based products

Commercial Miticidal Products*	Mortality Range (min-max)	N. Experiments	N. References
Amitraz	72% (1-100%)	98	17
Apitraz®	83% (70-100%)	22	4
Apivar®	66% (17-100%)	22	6
Mitaban®	>32%	1	1
Taktic®	70% (50-90%)	2	1

*Not reported:

- Method (petri dish, microapplicator, glass vial, apiarium, paraffin capsule, mason jar, plastic cup, laboratory cage),
- Dose ($\mu\text{g/mL}$, $\mu\text{g/mite}$, mg, μg , mL, mM, ppm, mg/cm², g of strip)
- Contact time (from 1 to 24h, not applicable for topic application)
- Assessment duration (from 1 to 49h)

● Amitraz Resistance Assessment

Commercial Miticidal Products*	Investigated population	Susceptible population	Quantitative Resistance Assesment
Amitraz	Czechia 2014	Czechia 2014	RR: 31.3, 7.5, 5.1
	Argentina 2009	Argentina 2007	RI: 3.9, 3.5, 3.7
	USA 2019	USA 2019	RR: 1.7, 3.9, 6.6, 2.6, 22.5 9.5 13.2, 7.9, 6.2, 3.2, 1.7
Apivar®	France 1998	France 1995	MLT: 24.9 min (1995); 57.6 min, 45.5 min, 37.8 min (1998)
Taktic®	Mexico 2004	Mexico 2000	RI: 2.3, 12.77, 8.56, 26.66
	Mexico 2006–2007	Mexico 2000	RI: 12.77, 8.56, 26.66

Note: RR = resistance ratio, RI = resistance index, MLT = mean lethal time.

● Genetic and metabolic resistance

- Maybe more than a single-nucleotide polymorphism (Marsky et al., 2024).
- To date, no significant evidence of metabolic detoxification.



... need more research at global scale!

Review

Sensitivity and Resistance of Parasitic Mites (*Varroa destructor*, *Tropilaelaps* spp. and *Acarapis woodi*) Against Amitraz and Amitraz-Based Product Treatment: A Systematic Review

Michela Bertola *  and Franco Mutinelli 



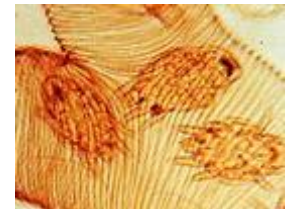
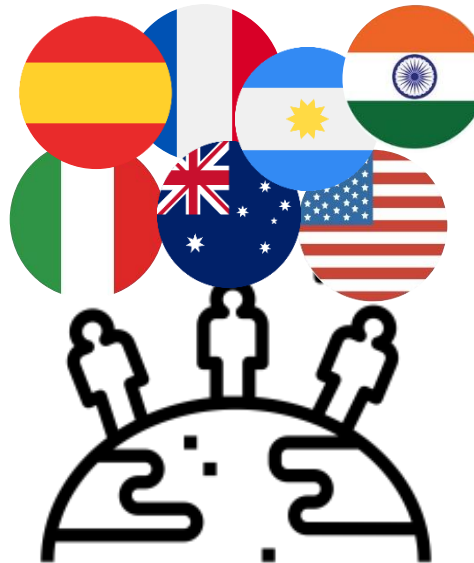
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Simple Summary: Amitraz resistance in *Varroa destructor* mites threatens global bee-keeping by reducing treatment efficacy and increasing colony losses. This study reviews 74 publications to map and analyze amitraz resistance in Varroa and other parasitic mites, such as *Tropilaelaps* spp. and *Acarapis woodi*. The findings reveal significant variability in resistance levels across regions, driven by inconsistent experimental protocols and environmental factors. Integrating bioassays, molecular diagnostics, and field tests is critical for accurate resistance detection. Standardized methodologies, national monitoring programs, and updated testing protocols are essential to managing amitraz resistance effectively, ensuring sustainable Varroa control and protecting global honey bee populations.

● Discussion

- Need of:
 - Standardized and validated protocol for amitraz-resistance laboratory assays
 - National programs for recording treatment efficacy and monitoring resistance outbreaks
 - Studies on other parasitic mites (Acarapis and Tropilaelaps)



● Questions?



**Thank you for
your attention!**

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<https://www.izsvenezie.com/reference-laboratories/beekeeping/>