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Effectiveness of synthetic acaricide treatments and development of resistance in *Varroa destructor* in Italy: historical overview and current considerations

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Abstract

The invasive ectoparasitic mite *Varroa destructor* represents the most significant health challenge for Western honey bees, *Apis mellifera*, and for apiculture worldwide. Its effective control is crucial to counteract the high colony mortalities it causes and ensure the sustainability of the beekeeping sector. To achieve such control, beekeepers rely heavily on the application of acaricide products to limit parasite loads. However, the effectiveness of these treatments is increasingly hindered by the growing issue of acaricide resistance. This problem is further intensified by the climate crisis: rising temperatures and the associated phenological shifts in honey bee colonies may limit the conditions under which non-synthetic acaricides can be applied, effectively narrowing viable control options to synthetic compounds. Here the authors review the current state of knowledge on *V. destructor* pharmacoresistance to synthetic acaricides in Italy, the country with the longest and most extensive history of investigations on the topic. To better capture resistance dynamics, relevant grey literature has also been included. We show how research findings on resistance have progressively influenced the development and adoption of miticides against *V. destructor* in Italy, and how rapidly these changes have occurred. The limitations of peer-reviewed scientific literature in capturing the full breadth of the phenomenon are also discussed. Like for many other countries, urgent additional research is needed in this field; despite the extensive historical body of work on *V. destructor* resistance, the current paucity of contemporary studies represents a serious concern for the future, limiting the ability to assess the scale of the problem and design effective strategies to counteract it.

Key Words

Acaricide resistance, *Apis mellifera*, beekeeping, honey bee health, *Varroa destructor*

Introduction

The invasive ectoparasitic mite *Varroa destructor* (Anderson & Trueman, 2000, *Varroa* hereafter) represents the most significant health challenge for apiculture worldwide (Traynor et al. 2020). Originally infesting the Eastern honey bee, *Apis cerana*, this mite shifted to the Western honey bee, *Apis mellifera*, in Asia during the mid-twentieth century (Oldroyd 1999). Since then, *V. destructor* has spread globally, compelling beekeepers to substantially modify management practices to prioritise mite control and ensure colony survival (Nazzi and Le Conte 2016; O'Shea-Wheller et al. 2025).

The life cycle of *V. destructor* is closely linked to that of *A. mellifera*, as the mite reproduces within sealed brood cells, where it is protected from adult bees and feeds on developing larvae and pupae (Traynor et al. 2020). Numerous strategies have been developed to manage *Varroa* infestations in honey bee colonies (Jack and Ellis 2021); however, the use of acaricide-based treatments remains the most

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common approach (Brodschneider et al. 2023). Among these, synthetic acaricides represented the first choice for beekeepers, due to their ease of application and affordability.

Unfortunately, this class of acaricides can cause severe side effects in honey bee colonies (Johnson et al. 2009), and may accumulate in and contaminate hive products (Milani 1999; Wallner 1999; Mullin et al. 2010). In addition, several factors limit their long-term effectiveness, most notably the development of resistance in *V. destructor* populations (Milani 1999; Elzen et al. 2000; Maggi et al. 2010; González-Cabrera et al. 2013). This parasite has in fact repeatedly adapted to the selective pressure imposed by synthetic compounds, particularly when these products are overused or improperly applied (Milani 1999). However, resistance in *Varroa* can be reversed, as this phenomenon is often associated with fitness costs, leading to a gradual decline in the frequency of resistance mites within the population once the selective pressure is removed (Denholm and Rowland 1992; Milani 1999).

Since the late 1980s, concerns about the aforementioned problems, together with growing awareness that alternative approaches are less prone to resistance development, have driven increasing interest in alternative control strategies (Rosenkranz et al. 2010). These include the use of veterinary medicinal products based on the so-called 'soft acaricides', like organic acids and essential oils, which currently are among the most widely used control options in Europe (Brodschneider et al. 2023). However, although these products offer a reduced risk of resistance development, their application presents important limitations: their efficacy is often restricted to specific phases of the honey bee life cycle (e.g., oxalic acid is effective only during broodless periods) or to narrow temperature ranges (e.g., treatments based on formic acid and essential oils). These constraints are particularly relevant in light of the ongoing climate crisis, as rising temperatures may shorten winter broodless periods (Neumann and Straub 2023), limiting the window of application of soft acaricides. In reaction to this, a future rise in the use of synthetic acaricides is therefore likely to be expected among beekeepers from regions most affected by climate change (e.g., Mediterranean area), as they can be used for prolonged treatments, even in the presence of brood, and at high temperatures. As such, it is thus prominent to assess the current status of *Varroa* resistance in these regions against synthetic acaricides, to prevent and control its emergence in the future.

Among Mediterranean countries, Italy stands out for its substantial body of research addressing this issue, as it was also the country where the first population of acaricide resistant *V. destructor* was detected (Colombo et al. 1993; Milani 1994, 1999). However, the data from these studies have never been comprehensively analysed together. In addition to peer-reviewed studies, a considerable amount of grey literature on this topic exists in this country, much of which has not been disseminated nor synthesised at the international level. Thanks to this type of literature, the different strategies put in place to control the spread of *V. destructor* in Italy and mitigate the damages from the onset of its invasion could be reconstructed.

By drawing from both international scientific literature and national grey literature, this review aims to provide a historical overview of the development of pharmacological resistance to synthetic acaricides in Italian *V. destructor* populations. We analysed 38 publications on *V. destructor* susceptibility and resistance to synthetic acaricides in Italy, from both scientific peer reviewed articles and grey literature, to widen the understanding of the phenomenon throughout the country, identify gaps in this research field and highlight the next steps.

Materials and methods

A systematic review was conducted to assess the resistance status of *V. destructor* mite to synthetic acaricides in Italy following established guidelines (Page et al. 2021). The key elements for this review included: (i) population: *Varroa* mites, (ii) context: Italy and (iii) outcome: the reduced efficacy of synthetic acaricidal treatments and the development of pharmacoresistance against these veterinary medicinal products. Complete methodology (research strings, eligibility criteria and PRISMA flowchart) is provided in Suppl. material 1. We considered all published studies that up to 1 October 2025 appeared in peer-reviewed journals indexed in PubMed, Web of Science Core Collection (WOS), Scopus, Google Scholar and CAB Abstracts. Moreover, given the major role that national beekeeping literature had in informing varroacide research in Italy during the '80s – '90s, we included those publications, reporting original data, as well to widen the scope of this review and provide a more complete picture on development and trends of *Varroa* resistance in Italy.

The screening process was conducted by the authors, who first classified all the studies from the initial literature search based on their titles and abstracts. When abstracts were unclear or lacked sufficient details, or when there was any uncertainty about the reported data, the entire study text was included for full evaluation. Each record was independently assessed based on pre-established eligibility criteria. Any disagreement was resolved through discussion and consensus among the reviewers. After retrieving the full texts, each reviewer extracted the relevant data, sourcing information from the text, tables, and figures, and entering them into predefined tables.

The extracted data were then cross-checked to ensure accuracy and consistency.

Results

Study selection and study characteristics

After the screening process, a total of 38 publications investigating chemical treatments against *Varroa* in Italy were included in the review. These investigated either the

reduced efficacy of synthetic acaricidal treatments (n = 31) and/or the development of pharmacoresistance against them (n = 9). The search strategy and the complete list of selected paper are listed in Suppl. material 1: tables S2, S3.

Sensitivity and pharmacoresistance status in *Varroa* mites in Italy

Relevant information regarding the loss of efficacy under field conditions and the emergence of pharmacoresistance, as reported in the selected publications, are summarized below. The findings are organized in chronological order and according to the pharmacological class of the active ingredient (see Tables 1, 2 and Fig. 1). For the purposes of this review, studies were classified as reporting either "reduced efficacy under field conditions" or "pharmacoresistance", according to the framework described by Milani (1995) and Bertola and Mutinelli (2025). Studies reporting a decrease in treatment efficacy under field conditions, without specific laboratory confirmation of resistance, were classified as "reduced efficacy", since suboptimal treatment performance may be influenced by several factors other than resistance. Conversely, studies were classified as assessing pharmacoresistance when resistance was specifically investigated under laboratory conditions using genetic or metabolic analyses and/or phenotypic assays. In phenotypic assays, evidence of reduced susceptibility was based on the comparison of the investigated mite population, suspected to be resistant, with a reference susceptible population, i.e., a population with no previous exposure, or documented susceptibility, to the active substance under investigation. In addition, the list of synthetic acaricidal commercial products used in Italy and their main characteristics is provided below in order to give a comprehensive overview of the options available to beekeepers in Italy during that period (Table 3).

Pyrethroids (tau-fluvalinate and flumethrin)

The first field studies on the effectiveness of tau-fluvalinate (commercialised as Apistan®), began in 1986–1987 in the Northeast Italian territory (i.e., Friuli Venezia Giulia region), when an effectiveness of approximately 92% was recorded, compared with the 99.6% initially claimed by the manufacturer (Milani and Barbattini 1989; Frilli et al. 1991).

In following years, lower effectiveness was reported in other regions as well, such as Emilia Romagna (Lodesani et al. 1990), Lombardy (Loglio and Plebani 1992; Colombo et al. 1993; Loglio 1993; Astuti et al. 1995; Lodesani et al. 2004), Sicily (Arculeo et al. 1993), Tuscany (Accorti and Luti 1994), Piedmont (Zeppegno et al. 1994), Sardinia (Floris and Prota 1993; Floris et al. 1995) and Trentino Alto Adige (Gufler and Wallner 1995). For example, in Lombardy, its efficacy was around 87% in 1991, and worsened in 1993 when it was recorded at about 70% (Lodesani 1996). This decline occurred after the veterinary services started distributingApistan® strips within this region (Loglio and Plebani 1992; Astuti et al. 1995; Lodesani 1996).

The first laboratory evidence of tau-fluvalinate resistance in Italian *Varroa* mites was reported in 1993, only four years after the introduction of pyrethroids for *Varroa* control (Colombo et al. 1993; Milani 1994). Specifically, the LC50 (200 mg/kg of tau-fluvalinate) for mites from Lombardy was recorded as being 10 times higher than that for mites from Friuli Venezia Giulia.

The following year, Milani (1995) reported a 20–50-fold increase in the LC50 of mites from areas where tau-fluvalinate treatments were no longer effective, compared with susceptible populations, as well as a 10–60-fold increase in the LC50 of other pyrethroids, i.e. flumethrin and acrinathrin. Laboratory bioassays also demonstrated cross-resistance in *V. destructor* between fluvalinate and other structurally related pyrethroids, including flumethrin and acrinathrin (Milani 1995).

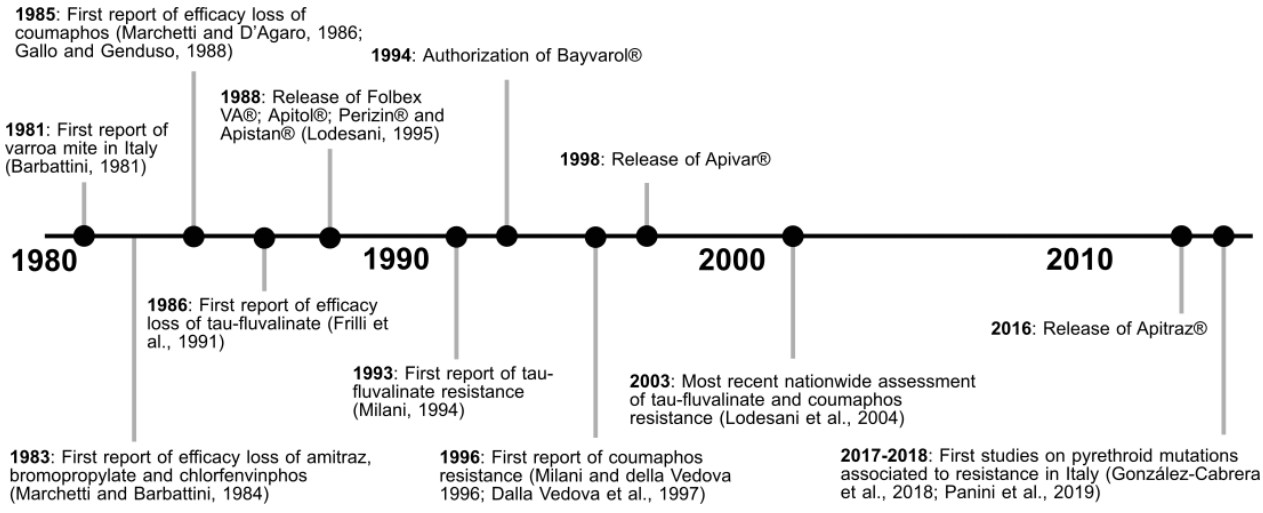

Figure 1. Timeline of release of authorised medicinal products against *V. destructor* in Italy and associated research findings over their efficacy and resistance.

Table 1. Loss of efficacy reports against hard acaricides in Italian *Varroa* populations ordered by pharmacological class and year of data collection.

Pharmacological class (active principle)	Commercial product (or active principle)	Mean efficacy % (min-max, if reported)	Region/s	Year	References
Formamidine (amitraz)	Taktic®	91.3	Friuli Venezia Giulia	1983	Marchetti and Barbattini 1984
	Bumetran®	From 22.73 to 2.52 ratio bee/varroa (*V. jacobsoni* population decreased of about 95%)	Sicily	1985–1986	Gallo and Genduso 1988
	amitraz	98 (fumigation); 90 (aereosol, applied once); 96 (aereosol, applied twice)	Emilia Romagna	1987–1989	Lodesani et al. 1990
	Apivar®	83.8	Sardinia	1999	Floris et al. 2001
Organochlorine (bromopropylate)	Folbex VA®	95.9	Friuli	1983	Marchetti and Barbattini 1984
	bromopropylate	96	Emilia Romagna	1987–1989	Lodesani et al. 1990
	Folbex VA®	73.6	Friuli	1991	Greatti et al. 1992–1993
Organophosphate (chlorfenvinphos)	Apiakaridim®	89.2	Friuli	1983	Marchetti and Barbattini 1984
	chlorfenvinphos	LC50 (µg/kg): 140.5 (151–130)	Friuli Venezia Giulia and Emilia Romagna	not reported	Milani et al. 2009
Coumaphos	Perizin®	97.86 (92.19–99.97)	Friuli Venezia Giulia	1985	Marchetti and D'Agaro 1986
	Asuntol®	From 13.81 to 2.69 ratio bee/varroa (*V. jacobsoni* population decreased of about 95%)	Sicily	1985–1986	Gallo and Genduso 1988
	Perizin®	95 (35–99)	Friuli Venezia Giulia	1986	Chiesa et al. 1987
	Perizin®	97.9	Friuli	1986–1991	Frilli et al. 1991*
	Perizin®	97.9 (92.2–99.9)	Friuli	1988	Barbattini et al. 1989*
	Perizin®	LC50 (ppm) = 21.3 (8.1–24.7) 26 °C; 8.9 (5.7–8.5) 32.5 °C	Friuli	1994	Milani and Della Vedova 1996
	Cekafix®	LC50 (ppm) = 30.3 (19.6–48.1) 26 °C; 19.4 9.8–29.2) 32.5 °C	Friuli	1994	Milani and Della Vedova 1996
	Perizin®	76 (36–80)	Emilia Romagna	1995	Lodesani 1996
	Perizin®	46 (28–88)	Lombardy	1998	Spreafico et al. 2001
	Perizin®	91.8	Aosta Valley	2000	Gerbelle et al. 2000*
Pyretrhoid (flumethrin)	Bayvarol®	99.7	Friuli	1986–1991	Frilli et al. 1991*
	Bayvarol®	99	Friuli	1989	Milani et al. 1989
	flumethrin	48 (4 strips); 68.6 (2 strips)	Sardinia	1997	Floris et al. 2001
Pyrethroid (tau-fluvalinate)	Apistan®	92.3	Friuli	1986–1991	Frilli et al. 1991*
	Apistan®	92.3 (63 days); 97.7 (4 months)	Friuli	1987–1988	Milani and Barbattini 1989
	tau-fluvalinate	93–98 (in wood); 98 (in PVC strips)	Emilia Romagna	1987–1989	Lodesani et al. 1990
	Apistan®	92.3 (83.3–99.6) 8 week; 97.7 (95.9–99.7) 16 week	Friuli	1988	Barbattini et al. 1989*
	Apistan®	99.7	Friuli	1991	Greatti et al. 1992–1993
	Apistan®	87.2 (group 1) and 90.5 (group 2)	Lombardy	1991	Loglio and Plebani 1992*
	Apistan®	95.45 (92.6–98.3)	Sicily	1991–1992	Arculeo et al. 1993*
	Apistan®	99.3	Sardinia	1992	Floris and Prota 1993*
	Apistan®	Hundreds or thousands of mites after the treatment	Lombardy	1992	Loglio 1993*
	Apistan®	55.71 (1992); 53.4 (1992)	Tuscany	1992–1993	Accorti and Luti 1994
	tau-fluvalinate	90.5	Piedmont	1992–1993	Zeppegno et al. 1994*
	Apistan®	99	Sardinia	1992–1994	Floris et al. 1995*
	Apistan®	44.5	Lombardy	1993	Lodesani et al. 1995
	Apistan®	≥ 90 only in the 36.7% of treated colonies	Lombardy	1993	Astuti et al. 1995*
	Apistan®	48.8 (4–89.5) Emilia; 44.5 Lombardy	Lombardy and Emilia	1993	Colombo et al. 1993*
	Apistan®	> 99	Sardinia	1994	Cabras et al. 1997
	Apistan®	some hives contained up to 1000 mites in spite of monthly Apistan treatments (one-fifth of apiaries exhibited resistance)	Trentino-Alto Adige	1994	Gufler and Wallner 1995*

Pharmacological class (active principle)	Commercial product (or active principle)	Mean efficacy % (min-max, if reported)	Region/s	Year	References
Pyrethroid (tau-fluvalinate)	Apistan®	29.7 (3–96)	not specified	1994	Faucon et al. 1995
	tau-fluvalinate	86.4 (4 strips); 92.2 (2 strips)	Sardinia	1997	Floris et al. 2001
Pyrethroid and essential oil	tau-fluvalinate (Apistan®) + thymol (Api Life Var®)	93.6 (88.21–98.89)	Friuli Venezia Giulia	2009	Frilli et al. 2009*

Table 2. Resistance report against hard acaricides in Italian *Varroa* populations.

Commercial product (or active principle)	Region(s)	Year	Reference
Tau-fluvalinate	Friuli Venezia Giulia and Lombardy	1993	Milani 1994
Acrinathrin, tau-fluvalinate and flumethrin	Friuli Venezia Giulia, Lombardy and Piedmont	1994	Milani 1995
Tau-fluvalinate	Friuli Venezia Giulia, Lombardy and Lazio	1995–1997	Trouiller 1998
Coumaphos	Emilia Romagna	1996	Della Vedova et al. 1997*
Tau-fluvalinate	Sardinia	1997	Floris et al. 2001
Tau-fluvalinate	Friuli Venezia Giulia	1997–2000	Milani and Della Vedova 2002
Coumaphos	Lombardy	1998	Spreafico et al. 2001
Coumaphos and Tau-fluvalinate	Lombardy, Piedmont, Veneto, Trentino Alto Adige, Friuli Venezia Giulia, Emilia Romagna, Marche, Abruzzo, Latium, Calabria, Sicily	2003	Lodesani et al. 2004
Tau-fluvalinate	Lombardy	2017–2018	Panini et al. 2019

Note: * Grey literature.

For this reason, although Bayvarol® (flumethrin) was authorized, it was never marketed in Italy, as evidence of pyrethroid resistance was already emerging at the time of its authorization.

Unfortunately, the emergence of resistance was accelerated by the continuous, and sometimes improper, use of pyrethroid-based acaricides, including failure to follow manufacturers' instructions and the widespread adoption of "do-it-yourself" practices (such as the use of homemade wooden strips impregnated with agricultural formulations of Klartan®, tau-fluvalinate 24% or Mavrik®) which greatly increased selective pressure on the mites (Frilli 1989; Bassand 1993; Loglio 1993; Lodesani 1996). This was further confirmed by subsequent reports of resistance in studies conducted between 1995 and 2000 in Friuli Venezia Giulia, Lombardy, Latium, and Sardinia (Trouiller 1998; Floris et al. 2001; Milani and Della Vedova 2002).

The most recent nationwide assessment of fluvalinate resistance was conducted by Lodesani et al. (2004). In this study, mite survival at a concentration of 200 µg/g did not exceed 27% and was below 10% in the great majority of cases (44 out of 50 samples). The distribution of resistance was highly heterogeneous, with higher levels mainly detected in specific areas of Lombardy, Emilia-Romagna, and Friuli Venezia Giulia. Overall, these results suggest that a general decline in the frequency of resistant mites had occurred in Italy at the time, consistent with a process of reversion following reduced use of pyrethroids (Milani 1999). However, two subsequent studies investigated the occurrence of mutations conferring pyrethroid resistance in Italian *Varroa* mites: in Lombardy (2017–2018), Panini et al. (2019) reported 11.1% homozygous resistant and 2.1% heterozygous individuals, whereas in Sicily, González-Cabrera et al. (2018) found a markedly higher prevalence, with 39% homozygous and 6.8% heterozygous genotypes.

Coumaphos

Perizin®, a coumaphos-based product intended for treatments during broodless periods and mainly applied during winter in Italy, was already known for its non-negligible residual toxicity to bees (Gashout et al. 2018), which limited its acceptance among Italian beekeepers. According to its summary of product characteristics, a slight bee mortality may be caused, i.e., the death of 100–150 honey bees after each treatment is expected. During the first years of use, coumaphos showed efficacy above 95% in several Italian regions, including Friuli Venezia Giulia (Marchetti and D'Agaro 1986; Chiesa et al. 1987; Barbattini et al. 1989; Frilli et al. 1991) and Sicily (Gallo and Genduso 1988). However, a modest but significant increase in tolerance to coumaphos was first detected in some areas of Emilia in 1995 (Lodesani 1996), and this loss of susceptibility was subsequently confirmed by laboratory assays the following year, with 1.5% of mites surviving exposure to the LC_{99} concentration (50 ppm of coumaphos) (Milani and Della Vedova 1996; Della Vedova et al. 1997).

This situation was further aggravated by inappropriate practices, including the use of empirical, extemporaneous formulations prepared with non-authorized products (e.g., Cekafix®) and the widespread adoption of homemade

Table 3. List and characteristics of synthetic acaricidal commercial products that in the publications analysed in this review were reported to be used in Italy.

Pharmacological class	Active principle	Commercial product name	Officially authorized for apiculture	Rapid (R) or prolonged (P) action
Organochlorine acaricides	Bromopropylate	Folbex®	Yes	R
		Folbex VA®	Yes	R
Imidazoles	Cymidazole	Apitol®	Yes	R
Organophosphates	Coumaphos	Perizin®	Yes	R
	Coumaphos	Cekafix®	No	
	Coumaphos	Asuntol®	No	
Pyrethroids	Tau-fluvalinate	Apistan®	Yes	P
	Tau-fluvalinate	Klartan®	No	
	Tau-fluvalinate	Mavrik®	No	
	Flumethrin	Byvarol®	Yes	P
Formamidines	Amitraz	Apivar®	Yes	P
	Amitraz	Apitraz®	Yes	P

strips impregnated with Asuntol®, a formulation containing the same active ingredient but exclusively approved for use in mammals (Della Vedova et al. 1997). These practices resulted in uncontrolled exposure of both mites and honey bees to coumaphos, increasing toxicological risks and intensifying selective pressure for resistance. Consequently, Perizin® lost efficacy while still maintaining a significant level of residual toxicity to bees. The latter was probably the main reason for the abandonment of this medicine for the winter control of *Varroa* mite infestation.

In 1998–1999, field and laboratory tests conducted by Spreafico et al. (2001) confirmed a loss of efficacy (only 46%) and increased tolerance of coumaphos (Perizin®) in *Varroa* populations from Lombardy and Friuli Venezia Giulia. In 2000, loss of efficacy was also reported in Aosta valley (Gerbelle et al. 2000).

Lodesani et al. (2004) reported moderate levels of resistance to coumaphos across Italy, particularly in situations involving improper use of coumaphos-based products not approved for apicultural use.

Due to the combination of reduced efficacy, safety concerns, residual toxicity, and regulatory non-compliance, Perizin® was permanently withdrawn from the Italian market in 2008.

Amitraz

The formamidine amitraz was widely used in the early years of *Varroa* control. Unfortunately, its use was improper, as several unauthorized amitraz-based products (such as Taktic®, Bumetran®, and Edrizar®) and homemade preparations were extensively applied (Frilli 1989; Loglio 1993; Lodesani 1996).

The first evidence of loss of in-field efficacy dates to 1983 (Marchetti et al. 1984) in Friuli Venezia Giulia and to 1985–1986 in Sicily (Gallo and Genduso 1988). By the end of the ’80 decreased effectiveness was also reported in Emilia Romagna and Sardinia (Lodesani et al. 1990; Floris et al. 2001).

The first published report of amitraz resistance in *V. destructor* dates back to Yugoslavia in 1991, after only four years of use (Dujin et al. 1991). Since then, field treatment failures and laboratory evidence of amitraz resistance have been reported worldwide (Bertola and Mutinelli 2025); however, to date, no published data on amitraz resistance in *Varroa* are available for Italy.

Bromopropylate

During the 1980s, bromopropylate seemed to maintain high efficacy in apiary, reaching 95.9% in Friuli Venezia Giulia and 96% in Emilia-Romagna (Marchetti et al. 1984; Lodesani et al. 1990).

In 1991, however, field trials conducted in the Trieste area (Friuli Venezia Giulia) showed a lower efficacy of Folbex VA® (73.6%) (Greatti et al. 1992) but no proper assessment of resistance was performed for this active ingredient in Italy. This product was soon withdrawn due to both the persistent residues in beeswax and the toxicity for operators as it was administered by fumigation.

Chlorfenvinphos

The first data on chlorfenvinphos effectiveness dated back to 1983, reporting approximately 90% of efficacy (Marchetti et al. 1984). To our knowledge, only one study assessed the susceptibility of Italian *Varroa* mites to chlorfenvinphos by determining the LC_{50} (Milani et al. 2009). The use of this active substance in apiculture has never been authorized but beekeepers made use of it in the 1990s following the emergence of resistance to pyrethroids (Gallina et al. 2006). However, this active ingredient is currently no longer authorized for use in many countries, including Italy, due to concerns related to toxicity and the presence of residues in hive products.

Discussion

The continuous reports of acaricide resistance worldwide, even in contexts where rotation of active ingredients is applied, highlight both the complexity and urgency of the current situation (Mitton et al. 2022). The development of resistance in *V. destructor* is driven by the interplay between biological determinants (i.e., mite genetics, physiology, and population ecology) and operational factors related to beekeeping practices, including treatment frequency, dosage, and application methods (Martin 2004; Sammataro et al. 2005; Hernández-Rodríguez et al. 2022). Ultimately, however, resistance dynamics are also constrained by the range of products available on the market. Since the first report in 1981, Italian beekeepers attempted from the outset to counter the spread of the mite by using amitraz-based products, mirroring the strategy adopted in Eastern European countries, where the parasite was present for longer (Koeniger and Fuchs 1989; Trouiller 1998). Soon after, several medicated formulations became available on the Italian market, including bromopropylate, cymiazole, coumaphos, tau-fluvalinate, thymol and flumethrin. Initially, synthetic acaricides were formulated as rapid-action treatments intended for use during broodless periods (Folbex®, Folbex® VA, a.i. bromopropylate, Apitol®, a.i. cymiazole, or Perizin®, a.i. coumaphos) but soon the available formulations shifted to products to be used in one prolonged treatment and intended for broodright colonies (Apistan®, a.i. tau-fluvalinate).

In this review, we identified six synthetic active ingredients whose use and effectiveness against *V. destructor* have been documented in Italy. A decline in efficacy has been reported for all of these compounds, with the exception of chlorfenvinphos, which, notably, was never authorized for use. In addition, our findings add to those of previous studies (e.g., see Wallner 1999 for pyrethroyds, Lodesani et al. 2008 for chlorfenvinphos) indicating that Italian beekeepers have resorted to the use of different unauthorized substances to control *Varroa*, likely reflecting both the limited availability of registered products at the time and the rapid loss of efficacy of those available. Moreover, at present, only two of the active ingredients used since the first years of *V. destructor* invasion, namely tau-fluvalinate and amitraz, remain available to Italian beekeepers as authorized veterinary medicinal products, since the other compounds were discontinued due to a combination of toxicity concerns and documented reductions in efficacy. This underscores the narrow range of available synthetic active ingredients for controlling *V. destructor* in Italy, constituting a significant vulnerability in the current control framework.

The historical patterns here reconstructed are particularly informative, as they show that the loss of efficacy is not an isolated phenomenon but a recurrent outcome following the introduction of synthetic acaricides, as is generally expected in the field of pests' chemical resistance (Siddiqui et al. 2023). Consequently, the active ingredients currently in use should still be considered at risk of reduced effectiveness over time. For instance, tau-fluvalinate already exhibited a decline in efficacy exceeding 10% within five years of introduction, reaching nearly a 30% reduction after approximately seven years.

Nevertheless, despite the widespread loss of effectiveness, research on *Varroa* pharmacoresistance in Italy has mostly focused on pyrethroids, leaving gaps in our understanding of resistance dynamics in other chemical classes, whose use was much more limited.

At the mechanistic level, resistance to pyrethroids has been identified with specific mutations associated with reduced target-site sensitivity (González-Cabrera et al. 2018). Studies on the reversion of pyrethroid resistance have shown that this process is inherently slow: the frequency of fluvalinate-resistant mites decreases by approximately one order of magnitude over three years, during which more than 30 mite generations may occur (Milani and Della Vedova 2002). Consequently, pyrethroid-based treatments may still retain some effectiveness if applied within an adequate rotation In parallel, similar resistance mechanisms to those observed for pyrethroids have also been documented for amitraz in other countries. For instance, at the molecular level, two amino acid substitutions (N87S and Y215H) in the β2-adrenergic-like octopamine receptor (Octβ2R), the primary target site of amitraz, have been associated with treatment failures in France and the United States (Hernández-Rodríguez et al. 2022; Rinkevich et al. 2023; Bahreini et al. 2025). In Spain, Hernández□Rodríguez et al. (2025) identified an additional point mutation (F290L) in the same receptor, also linked to amitraz resistance. Further point mutations have been reported in other geographical areas as well, including T115N in South Korea and Y337F in Belgium and Turkey (Inak et al. 2024; Lee et al. 2025). Nevertheless, according to Marsky et al. (2024) the development of amitraz resistance likely involves multiple genetic variants and complex evolutionary processes. In this context, the lack of studies investigating amitraz resistance in Italy represents a critical knowledge gap. In particular, it remains unclear if any mutations are present in Italian *V. destructor* populations, limiting our ability to assess resistance risk and to implement targeted monitoring and management strategies.

An additional aspect emerging from this review is the critical role of grey literature, which often anticipates trends later documented at the international level (Mitton et al. 2022). As highlighted in this review, early signals of resistance development or treatment failure have first appeared in technical reports, local surveys, and institutional documents before being captured in peer-reviewed publications. Moreover, national grey literature enables the evolution of *Varroa* management within a country to be tracked, providing valuable insights into how control strategies have adapted over time in response to resistance emergence, regulatory changes, and local beekeeping practices. This is particularly relevant in a pharmacoresistance framework, as it allows the identification of early warning signals and strategic shifts that may guide future interventions. Moreover, given the transboundary

nature of beekeeping practices, evidence generated in one country may contribute to informing and strengthening preventive strategies in neighbouring regions (Faucon et al. 1995).

Finally, the historical reconstruction of control strategies, treatment inefficacy and resistance patterns is indispensable for meaningful comparison with the present situation. Without this retrospective perspective, the interpretation of current resistance levels would be severely limited by the lack of baseline data. In this sense, long-term national monitoring programs, supported by robust pharmacovigilance and surveillance frameworks, are essential to promptly identify treatment failures, ensure the correct use of veterinary medicinal products, and raise awareness of the economic, environmental, and ecological impacts of varroosis. Strengthening these systems will contribute not only to improved *Varroa* management but also to the broader goal of safeguarding honey bee health and ecosystem services.

More than forty years after the first detection of *Varroa* in Italy, its effective control remains a major challenge. Nowadays, sustainable management of infestations requires a planned and integrated approach, combining chemical treatments with biotechnical control methods (Jack and Ellis 2021). Previous studies have already emphasized the value of regular surveillance programs aimed at monitoring mite susceptibility to acaricides (Spreafico et al. 2001; Panini et al. 2019), as early detection of resistant populations is crucial to minimizing economic losses (Milani 1999). However, our findings highlight the major lack of research on this topic in Italy.

Conclusions

In the absence of updated and robust data on pharmaco-resistance status of *Varroa* populations, it will not be possible to implement comprehensive, evidence-based management strategies for the effective and sustainable control of this mite. Addressing this gap is therefore essential to ensure the long-term efficacy of available control tools. The implementation of integrated resistance management strategies includes the rotation of active ingredients with different modes of action, the incorporation of biotechnical measures, regular assessment of infestation levels both before and after treatments and the systematic monitoring of mite populations through bioassays and molecular tools. Overall, strengthening surveillance, improving management practices, and promoting an integrated approach are key steps to mitigate resistance development and safeguard the sustainability of apiculture.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
Regarding the use of AI in the preparation of this manuscript, the authors declare the following: GPT
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Author contributions

MB and FM conceived and designed research. MB conducted the review. All authors wrote, revised and approved the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

Supplementary material 1

Additional information

Authors: Michela Bertola, Arrigo Moro, Franco Mutinelli
Data type: docx
Explanation note: **table S1.** String construction. **table S2.** Search strategy. **table S3.** List of elected papers.
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