Apidologie (2021) 52:1305–1333

https://doi.org/10.1007/s13592-021-00903-4

Review article

Three pillars of *Varroa* control

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Received 4 September 2020 – Revised 13 August 2021 – Accepted 1 November 2021

Abstract – The beekeeping sector is facing many challenges. One of the greatest is maintaining healthy colonies that produce high-quality products without any residues of veterinary medicines and with low environmental impact. The main enemy is the ectoparasitic mite *Varroa destructor*, the most significant honeybee pest and a key factor in high colony losses worldwide. In the previous four decades, three pillars of *Varroa* control have crystallized to be essential for sustainable management: apitechnical measures, chemical treatments, and resistant stocks of honey bees. In the long term, the latter is probably the most sustainable as it is a step to self-sustaining populations of feral and managed colonies. We recognize the significance of progress in knowledge of all three pillars to conquer *Varroa* and of their successful usage in accordance with local and global conditions and capabilities. In this review, we present a possible integration of the components of the three pillars of *Varroa* control strategies in the light of sustainable beekeeping and provide their linkage to the production of high-quality and safe honeybee products and maintaining healthy colonies.

***Varroa destructor* / *Varroa* treatment / control methods / low environmental impact / effectiveness / honeybee product safety**

1. INTRODUCTION

The Western honey bee *(Apis mellifera)* is of great economic importance, both due to the value of its pollination services and the value of honeybee products. In recent decades, however, huge colony losses have been reported worldwide (Neumann and Carreck 2010). The reasons for colony losses are diverse: a lack of forage diversity and the intensive use of pesticides are often cited, along with honeybee diseases. Varroosis, among the latter, caused by *Varroa destructor* (Anderson and Trueman 2000), plays a crucial role in honeybee colony mortality. Members of the genus *Varroa* are obligatory ectoparasites of different species in the genus *Apis*. Initially, *V. destructor* was a parasite of *Apis cerana Oud.* (Anderson and Trueman 2000). In the twentieth century, it shifted hosts to parasitize *A. mellifera* (Oldroyd 1999; Roberts et al. 2015). At least six haplotypes of *V. destructor* are known (de Guzman et al. 1998), but only two shifted from *A. cerana* to *A. mellifera*: The

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Manuscript editor: Yves Le Conte.

Korean haplotype is present worldwide and is considered more pathogenic; the Japan/Thailand haplotype, which was reported in *A. mellifera* colonies only in North and South America, Japan, and Thailand, is considered less virulent (Anderson and Trueman 2000; De Guzman et al. 1998).

In the meantime, the sister species *Varroa jacobsoni* has also been reported to parasitize *A. mellifera* in Papua New Guinea (Roberts et al. 2015).

The life cycle of *Varroa* is characterized by dispersal and reproductive stages. In the dispersal stage, the adult mites feed on the fat body on the adult bee's abdomen's ventral side hidden under the sternites (Ramsey et al. 2019). The reproduction phase is in capped worker and drone brood. Prior to the capping of honeybee larvae (5th instar larva), the adult (mother) mite invades the cell with developing larvae. Approximately 70 h after cell capping, the mother mite lays the first egg, which is normally an unfertilized male egg due to haplodiploid sex determination. Eggs are laid in 30-h intervals. Up to six eggs per female mite are considered normal. As reproduction occurs only in capped brood, males start to reproduce as soon as young mature females arrive on the mating site inside the closed brood cell (Rosenkranz et al. 2010). In addition to the direct negative effect on honey bees through its feeding on the fat body (Ramsey et al. 2019), which impacts the immune system and leads to smaller body size and shorter life span, *Varroa* is also a vector for viruses (deformed wing virus, acute bee paralysis virus, Kashmir bee virus, Israeli acute paralysis virus, Sacbrood virus), thus reducing the vitality of the entire colony (Boecking and Genersch 2008). Drones parasitized during larval development stages have reduced chances to mate and infested colonies produce fewer swarms (Duay et al. 2002; Fries et al. 2003; Villa et al. 2008). *Varroa* affects honeybee colony at different levels (Noel et al. 2020). It impacts individuals in the brood and adult stage (workers, drones), at the colony level (swarming, overwintering success, virus contamination), and the population level (spreading to nearby colonies, colony losses) (Villa et al. 2008).

In this review, we give an overview of available techniques of *Varro*a management, presented as three groups (or "pillars"). We suggest possible combinations of these three pillars as an approach of "integrated *Varroa* control" to sustainably address and overcome the current topical issues of existing control methods (e.g., mite resistance, acaricide residues in hive products, and increasing virulence of the mites).

2. LEGAL REQUIREMENTS IN CASE OF VARROOSIS IN THE EUROPEAN UNION

The term "varroosis" is defined as a disease of insects from the genus *Apis* caused by mites of the genus *Varroa*, primarily *V. destructor* (World Organisation for Animal Health, OIE 2019).

Varroosis is one of six diseases of bees, listed in the OIE *Terrestrial Animal Health Code*. OIE member countries and territories are obligated to report its occurrence (https://www.oie.int/en/disease/diseases-of-bees/).

The appearance of the disease is strongly diverse, depending on the infestation level and secondary infections (Boecking and Genersch 2008). Typical clinical signs of infestation are a patchy brood nest with empty cells interspersed between capped brood cells, bees with crippled wings and abdomen, and a sudden collapse of the colony. This pattern of signs is also referred to as a parasitic mite syndrome (Shimanuki et al. 1994).

Varroosis is a global problem. Even Australia, where no established *Varroa* population has been recorded yet, is affected by incursions from time to time (Queensland government, Department of Agriculture and Fisheries 2020). Individual countries handle the *Varroa* infestation in different ways according to their specific veterinary legislation. In the EU, varroosis is a listed animal disease (Commission Delegated Regulation (EU) 2018/1629, Annex II to Regulation (EU) 2016/429). National regulations may be in force in different EU member states on how to proceed in an outbreak of varroosis (e.g., notification to the authorities in Austria if a threshold of 30% of hives already dead or likely to die. In Italy, the veterinary authority may request the destruction of the hives in case of severe varroosis).

In general, honey bees are classified as food-producing animals. Therefore, veterinary medicinal products (VMPs), such as varroacides, must be scientifically evaluated according to human food safety requirements (e.g., Regulation (EC) No 470/2009 in the EU or specific regulations in force in other countries). In addition, EU maximum residue limits (MRLs) for residues of pharmacologically active substances in honey are in force and listed in Commision Regulation (EU) No 37/2010. For some substances (e.g., amitraz and coumaphos), an MRL has been established, while for other substances, evaluation has demonstrated that no MRL was required to protect food safety (flumethrin, oxalic acid, and tau-fluvalinate). MRLs of VMPs must be respected when treating varoosis. Products that have not been assessed as safe according to these requirements can neither be authorized nor be used otherwise for food-producing animals (Commision Regulation of European Commission 37/2010).

If prohibited substances according to EU Regulation (annex of Commision Regulation No 37/2010, Table II) are found by residue analyses, the honey or other bee products are not marketable in the EU, regardless of the level of residues. Sporadic evidence of unauthorized substances in honey (e.g., rotenone and bromopropylate; Nguyen et al. 2009) or in beeswax (chlorfenvinphos; Calatayud-Vernich et al. 2018) are indications for possible illegal use in *Varroa* management or the lack of awareness of beekeepers of the need to comply with legal requirements.

In most countries around the world, active ingredients and applied preparations must be approved and registered (e.g., as VMPs) by the competent authorities before they can be used legally in honeybee colonies. Also, restrictions on use and precautions (e.g., withdrawal period after treatments) to keep residues below the maximum residue limits in honeybee products must be respected. Since the EU is recognized as the world's major importer of honey (European Parliament 2018), honey-exporting countries are obliged to meet these requirements (e.g., Commission Regulation (EU) No 37/2010).

3. THREE PILLARS OF *VARROA* MANAGEMENT STRATEGIES IN THE EUROPEAN UNION

Any *Varroa* management strategies and treatment regimens should be based on the regular monitoring of the infestation levels of the mite in the colonies. For the international standard methods of diagnostic techniques, see Dietemann et al. (2013), Rosenkranz et al. (2010), Gregorc and Sampson (2019), and Roth et al. (2020). In addition, regular monitoring for the resistance of *Varroa* to the active ingredients used in chemical treatments should be carried out—according to the instruction manual before the application—to be able to react adequately, e.g., no application, regular alternation between products with different modes of action, and the integration of apitechnical measures for *Varroa* control (Roth et al. 2020).

To keep it simple for the practical beekeeper, we grouped the different *Varroa* control methods that are in practical use into three pillars (groups): (a) Apitechnical measures (which include beekeeping techniques and the use of specific biological relationships between *Varroa* and honey bees, (b) chemical treatments, and (c) breeding of resistant stock. In Sect. 4, we gathered methods that have already shown promising results in laboratory trials but lack on-field application.

To address the main problems of current *Varroa* management practices, a combination of methods from all three pillars may result in the most sustainable answer (Fig. 1). All the components of the three pillars are summarized in detail in the tables in the Appendix.

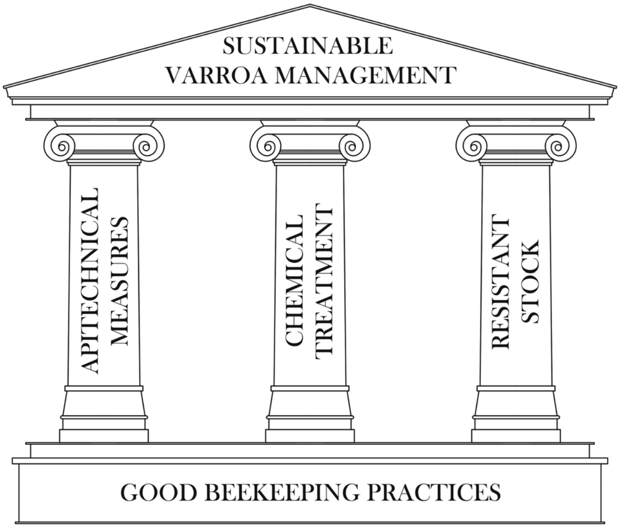

Fig. 1 Three pillars of sustainable *Varroa* management strategies in the EU

3.1. The first pillar—apitechnical measures

Apitechnical measures are applied to impede the increase of mite numbers in the colony, either without chemical treatment or to support and increase the efficacy of chemical treatment. Thus, they could help to reduce the number of necessary applications of VMP's. Their greatest advantage is that they can also be used in periods of nectar flow when chemical treatments are not allowed or not recommended in order to prevent residues in hive products.

However, applied alone, apitechnical measures are often not sufficient to keep the infestation level under the damage threshold during the whole season without additional measures (e.g., application of registered VMP's). The most used apitechnical measures are brood removal, drone brood removal, trapping comb, queen caging, thermotherapy, and use of screened bottom boards. More details about apitechnical measures could be found in the table in Sect. 7.1 under Appendix.

3.1.1. Brood removal, trapping comb, queen caging, and drone brood removal

As drone brood is more attractive to *Varroa* than worker brood is—if both are present in a colony (Fuchs 1990)—removing and destroying drone brood in a planned cycle eliminates mites during the drone-rearing period. After capping, the drone brood must be removed from the colony before the adult drones and mature *Varroa* hatch. This procedure can be performed several times per season (Charrière et al. 1998, 2003).

The removal of capped worker brood is similar but less effective than drone brood removal. As a standard procedure to establish mating nucs (Morse and Hooper 1985; LLH Bieneninstitut Kirchhain (2015)), a recommended apitechnical method is also to reduce the *Varroa* population in the donor colony (Liebig 1998).

Artificial brood interruption by total brood removal or caging of the queen is a method of depriving *Varroa* of the opportunity to hide and reproduce in brood cells. Thus, the mites are "forced" to remain in the phoretic stage

(Underwood and López-Uribe 2019; Büchler et al. 2020), where they are accessible for self- and social grooming behavior or chemical treatments. Total brood removal (TBR) immediately eliminates all mites and viruses that are in infested bee larvae. The TBR technique does not set the queen at risk of being rejected and enables removing large amounts of infectious material at once. Simultaneously, the colony can be treated effectively with acaricides. The use of TBR reduced the treatment costs (Mancuso et al. 2020) but increased the costs of feeding and workload per colony. In some cases, even the loss of honey crop was recorded (Mancuso et al. 2020).

With the trapping comb technique, the *Varroa* is trapped in a comb of worker brood. The queen must be confined on one comb, using a queen excluder cage. Every week, this comb is replaced by a new one, and the queen is relocated onto this new comb inside the queen excluder frame. The comb with uncapped larvae is placed in the hive and attracts *Varroa*, because it is the only comb in the colony with larvae of the right age for invasion. After capping, this brood comb is removed and destroyed (freezing, melting). The procedure is repeated three to four times in a row (Maul et al. 1988). The trapping comb technique is very effective and can reach an efficacy of 95% if it is done correctly with enough worker cells available to the queen (Beetsma et al. 1999; Calis et al. 1999; Charrière et al. 2003; Engels et al. 1984). The disadvantages of this method are the possible attenuation of the colony and labor intensiveness (Engels et al. 1984). An important aspect of this method is the starting point, which must be shortly before the peak of colony development is reached (May to June, depending on location) to avoid a decrease in spring and summer honey crop (LLH Bieneninstitut Kirchhain 2010).

Queen caging is a technique of brood interruption, in which the queen must be caged for 25 days until all the worker and drone brood has emerged. After 25 days, no brood is left and *Varroa* is forced to remain phoretic on the bees (Gregorc et al. 2017). Queen caging alone, without any VMPs, can reduce the *Varroa* population by up to 40.6% (Giacomelli et al. 2016). The main cause of increased mortality of *Varroa* is thought to be an overly long phoretic phase and the inability to reproduce. Queen caging in combination with "soft" VMPs, such as oxalic acid or thymol (see below), increases the efficacy up to 97% (Giacomelli et al. 2016).

However, confining a fully laying queen for 25 days in a small cage can cause severe negative side effects on the colony (e.g., rearing of emergency queen cells) and the queen itself (queen death inside the cage, rejection of the queen, or supersedure after release). In a 2-year trial in Austria, queen caging and subsequent oxalic acid trickling in summer resulted in the loss of 33 out of 193 queens (17.1%). In the alternative group (140 queens uncaged plus two applications of formic acid in colonies with brood), only 3 queens (2.1%) were lost (Ribarits et al. 2020). Jack et al. (2020) reported that caging the queen for brood interruption in the early fall, combined with oxalic acid sublimation, led to high mortality of colonies and insufficient *Varroa* control.

3.2. The second pillar—chemical Varroa control methods

This approach is based on the use of different chemical compounds inside beehives or applied directly onto bees to kill *Varroa* as effectively as possible. In the EU, according to Commission Regulation (EU) No 37/2010 (see annex of Commision Regula 37/2010, Table I), the following substances are, in principle, allowed to be used on honey bees: formic acid, oxalic acid, lactic acid, thymol, eucalyptol, menthol, camphor (all without MRL required), amitraz (MRL: 200 µg/kg honey), coumaphos (MRL: 100 µg/kg), tau-fluvalinate (no MRL required), flumethrin (no MRL required); Commission Regulation (EU) No 37/2010,). However, their approval as registered preparations by the respective competent authorities is also required before they could be used legally.

In the USA, potassium salts of hops beta acids (K-HBAs) are approved for *Varroa* control (no MRL required, because ingredients are classified as GRAS substances, which are Generally Recognized as Safe; EPA 2015a, b).

When applying any of these VMPs, the label must be followed strictly (e.g., time and maximum number of applications, withdrawal periods; mite resistance management, etc.) to ensure the efficacy and safety of bee products and to comply with the law. Additionally, in some countries (e.g., Austria, Slovenia), statutory requirements are in force, requiring that records must be kept for any use of VMPs on honeybee colonies.

For *Varroa* control, officially approved active ingredients in VMPs are either substances not occurring naturally in honey (e.g., pyrethroids: tau-fluvalinate, flumethrin, acrinathrin; amidins: amitraz; organophosphates: coumaphos; halocarbon compounds: chloro-, bromopropylate) or natural components of honey (e.g., organic acids: formic-, oxalic-, lactic acid; essential oils: thymol, menthol, camphor, eucalyptus oil). It should be noted that this list does not reflect the current approval status in general. This may differ from country to country, and there may be bans or time limits on approval for some active ingredients.

In practice, beekeepers often simply denominate varroacides as "soft" (or "environmentally friendly") if they contain active ingredients that are found in nature and often also are natural compounds of honey (BBVA 2017; Rosenkranz et al. 2010). However, this designation does not mean that these substances are generally safe for bees and users in every respect. Therefore, appropriate personal protective equipment must be used in their application according to safety instructions on the label.

In contrast, other varroacides are called "hard" (or "synthetic") as they contain synthesized proprietary chemicals that do not occur naturally in honey (BBVA 2017; Rosenkranz et al. 2010). However, the terms "soft" and "hard" are arbitrary. Furthermore, the active substances of both groups are synthesized by pharmaceutical companies in industrial processes and not extracted from natural raw materials, except hops beta acids, which are extracted from plants.

3.2.1. Hard acaricides

The beekeeper's denomination "hard" refers to the fact that these acaricides contain synthesized proprietary chemicals and not to their effect on the bees or the user. Amitraz, coumaphos, and the pyrethroids flumethrin and tau-fluvalinate are some of the most frequently used synthetic acaricides in approved VMPs in Europe and worldwide. Examples of some compounds used in the past at the beginning of chemical *Varroa* control but currently no longer approved or registered in the EU are bromopropylate (substance group: organobromine compound; acts as acetylcholine esterase inhibitor; Mehlhorn 2008) and cymiazole (substance group: amidins, acts on octopamine receptors; Mehlhorn 2016). Further details about the mode of action, efficacy, application mode, and possible side effects could be found in the table in Sect. 7.2 under Appendix.

3.2.1.1. Resistance of mites To date, resistance has been reported for pyrethroids, amitraz, and coumaphos in many countries (Roth et al. 2020; Rosenkranz et al. 2010; Lodesani et al. 1995; Hubert et al. 2014; Millán-Leiva et al. 2020; Rinkevich 2020; Moosbeckhofer and Trouiller 1996; Goodwin et al., 2005; Mozes-Koch et al. 2000).

For tau-fluvalinate and flumethrin resistance, several single mutations in the voltage-gated sodium channel have been identified (Gonzalez-Cabrera et al. 2013, 2016; Hubert et al. 2014). Resistant mites cause severe problems for the beekeepers, including heavy losses of colonies due to the decreasing efficacy of VMPs used. In the case of resistant mites, a management concept must be established with regular changes of applied VMPs. Due to the possibility of cross-resistance (e.g., between pyrethroids) or multiple resistant mites, a switch to agents of another substance class with a different mode of action is strongly recommended. Therefore, early detection of the development of mite resistance is crucial. This could be difficult in practice, because the level of resistance could be different between colonies in the same apiary, between apiaries of the same beekeeping operation, between local regions or countries, and from one season to another

(Hernández-Rodríguez et al. 2021; Millán-Leiva et al. 2020; Rinkevich 2020). The main reasons for such differences between regions or countries are the types of legal and illegal VMPs used, the duration of their application, the accumulation of residues in beeswax, a lack of resistance management concept, or the international trade with honey bees and queens. Within or between beekeeping operations, resistant mites are exchanged by swarms, robbing or drifting bees, hive movements, or selling and purchasing of bees and queens. Having an insight into the status of resistance in honeybee colonies is important for the selection of an appropriate and effective VMP for the next treatment. For example, the CMDv-list of authorized bee products (CMDv 2021) restricts the indication to non-resistant mites as follows: PolyVar® Yellow: "For the treatment of varroosis in honey bees caused by flumethrin sensitive *Varroa destructor* mites." Similar statements could also be found on Apitraz® and Apivar®.

Consequently, for safety reasons, these products could not be recommended for use in an apiary in a case of unknown resistance status of the mites. In addition, the instructions for use of the respective registered preparations recommend checking the acaricide susceptibility or resistance at the regional level by using biotests or molecular biological methods (PCR). However, this is not feasible in practice for a beekeeper! In the case of existing resistance, the product affected should not be applied. These recommendations are more of a theoretical value for the practical beekeeper because different countries usually do not have systematic screening for varroacide resistance. To the best of our knowledge, we are not aware of any laboratories or institutions that offer such resistance tests commercially for beekeepers. High colony losses or treatment failures had been a reason to carry out resistance tests within the scope of research projects in Spain (Hernández-Rodríguez et al. 2021) and in the USA (Millán-Leiva et al. 2020). For the active substances amitraz and flumethrin, the instructions for use recommend using them only as part of an integrated *Varroa* control program and in alternation with other active substances.

3.2.2. Soft acaricides

From the beginning, these substances have been the main backbone for *Varroa* control in organic beekeeping, in addition to the employment of apitechnical measures. The development and spread of resistant mites and residues of fat-soluble active ingredients in bee products have increased the interest and use of VMPs based on organic acids and essential oils, also in commercial beekeeping.

The denomination "soft" refers to the fact that these active ingredients contain chemicals that are found in nature and are often components of honey. However, it does not imply that they are, in general, without risk for honey bees or the user when applied. Apart from hop beta acids, they are also synthesized by the chemical industry.

Representatives of organic acids are formic, oxalic, lactic acid, and hop beta acids. Essential oils are thymol, menthol, camphor, and eucalyptol. Further details about the mode of action, efficacy, application mode, and possible side effects of soft acaricides may be found in the table in Sect. 7.2 under Appendix.

Some of these so-called soft acaricides are easy to use for the beekeeper and of low risk for the bees and the brood (e.g., registered products with thymol; blends of thymol with some other essential oils; oxalic acid; lactic acid; potassium salts of hop beta acids). However, others (e.g., formic acid, oxalic acid) require both protective equipment during handling and application as well as the skill and experience in application and have only a very small safety margin between high effectiveness against *Varroa* on the one hand and the avoidance of bee and brood damage on the other. Some types of active ingredients depend on their efficacy on external (e.g., ambient temperature) and internal factors (e.g., colonies with or without brood; evaporation rate per time; placement inside the hive).

Even though these active ingredients are safe and no MRLs are required for honey, their use according to label as well as off-label can lead to residues in honey (Bogdanov et al. 1998a, b; Bogdanov et al. 2002; Bollhalder 1998;

Moosbeckhofer et al. unpublished), which could be organoleptically perceived by the consumer (e.g., the taste and flavor of thymol). After application of one registered and two homemade thymol-based preparations, Serra Bonvehi et al. (2016) detected thymol residues in 86 to 90% of honey samples (mean 0.78–1.18 µg/g). In 11% of the samples, the concentration of thymol exceeded the taste threshold (1.20 µg/g), with a negative effect on the sensorial quality of the honey. By applying organic acids, the threshold for free acid of 50 milli-equivalents per 1000 g of honey must not be exceeded (Council Directive 2001/110/EC 2001).

Some lipid-soluble active ingredients (e.g., essential oils) can accumulate in both beeswaxes as well as in honey. Bogdanov et al. (1998a, b) showed that thymol did not disappear by melting of the combs, but the level of thymol decreased from comb and foundation when they were exposed to the air during storage or in the hive. Following an autumn treatment, the thymol residues in next spring honey were on average 0.15 mg·kg^{-1} ($n=29$) and below the taste threshold of 1.1 and 1.6 mg·kg^{-1} in black locust (*Robinia pseudoacacia*, also known as *Robinia*) and rape honey, respectively. The water-soluble organic acids do not leave residues in the wax. Concerning the honey, using formic acid during a honey flow can increase its content in the honey from treated colonies above the natural level of untreated colonies of the same flow (Moosbeckhofer, unpublished results). There are no reports of mite resistance against soft acaricides. A possible explanation is that the organic substances act in an unspecific way or on more than one target site. The action on more than one target or by physico-chemical effects makes resistance to these actives less likely to develop (German 2019). Nevertheless, a regular change of preparations with different modes of action is recommended as a precautionary measure.

In contrast, synthetic acaricides act in a very specific way on target sites of the mites. Consequently, even mutations in single target sites can lead to resistance (González-Cabrera et al. 2013; González-Cabrera et al. 2016; Hubert et al. 2014; Millàn-Leiva 2020).

3.3. The third pillar—selective honeybee breeding for resistance to Varroa

The search for resistant honey bees has been ongoing since the 1980s and continues in Europe and throughout the world. Resistance against *V. destructor* has been reported from different regions and honeybee species (see recent reviews by Locke 2016; Oddie et al. 2017; Guichard et al. 2020; Le Conte et al. 2020; Mondet et al. 2020; Spivak et al. 2021).

By natural selection, *Varroa* resistance developed in *A. cerana* colonies in Asia, but also in some populations of *A. mellifera* subspecies (Brazil: Africanized honey bees; Africa: *Apis mellifera scutellata*; *Apis mellifera capensis*; France: bees from Le Mans, Avignon; Norway: Østlandet bees; USA: Arnot Forest bees; Russia: Primorsky bees).

By artificial mass selection, resistance was achieved in Sweden (Gotland bees), France (Kefuss bees), and the Netherlands (Blacquière bees), using the survival of the colonies as a selection criterion. This approach was called the "bond test" ("live and let die!") in Sweden and France and the "Darwinian black box" selection for resistance in the Netherlands, because the underlying mechanisms of resistance remain unclear.

Another approach is selective breeding, meaning the genetic selection on chosen characters, such as grooming behavior (GB), hygienic behavior (HB), mite reproduction (MR), and suppressed mite reproduction (SMR), which are mainly attributable to *Varroa* sensitive hygiene (VSH; Harris 2007), postcapping stage duration (PSD), recapping (REC), reduced mite population development (MPD) and some other traits in breeding programs (reviewed by Le Conte 2020). It must be considered that these resistance traits and their interactions among mites, colonies, and environmental conditions play a crucial role in resistance to *Varroa*.

The main goals in the breeding of mite-resistant honey bees are the expected benefits: reduced colony losses, lower workload and the reduction of treatment costs for beekeepers, food security due to assured pollination services, and high-quality hive products.

Obtained knowledge and reproductive sources were the basis for selective breeding of *Varroa*-resistant honeybee lines in North America (reviewed by Rinderer et al. 2010) and Europe (reviewed by Büchler et al. 2010; Oddie et al. 2017). An overview of different genetic stocks (description of the behavior, institution that selected or imported stock, mite life stage affected) to reduce *Varroa* loads is presented by Cornell University, CALS Beekeeper Tech Team (n.d.).

However, in order to include selection for *Varroa* resistance successfully into breeding programs, close cooperation between all stakeholders within and around a breeding program is necessary (Uzunov et al. 2017). The interest and preference of beekeepers for resistant bees as a part of breeding goals are important and should be considered, as well as proper management to assure successful collective selection to enable the sustainability of such breeding programs (Guichard et al. 2019). Another important issue is the availability of resistant stock on the market (queens or queen cells). This will become a bottleneck in the future. Therefore, Le Conte et al. (2020) recommend the robust cooperation of beekeepers and breeders and the development of "effective infrastructures for the promotion of *Varroa*-resistant and commercially attractive honeybee stocks in the EU."

Simultaneously, an additional challenge arises from the fact that several subspecies of *A. mellifera* with their different ecotypes are native to different regions of Europe. Their preservation for the future is not only a matter of species conservation but also of practical beekeeping interests. Meixner et al. (2014) and Büchler et al. (2014) found in a comprehensive field experiment in different countries with different ecotypes of honey bees that the vitality of the local bees was higher compared to the non-local ones. This indicates that breeding bees from the local populations can lead to more sustainable beekeeping. Therefore, selection for mite resistance should also be based on local bees and not only on the introduction of resistant bee lines from external sources, selected under different environmental conditions or different mite populations.

There is growing interest in the development and utilization of methods that employ genotyping and taking advantage of marker-assisted selection (MAS) in the breeding and selection of honey bees.

The utilization of marker-assisted selection (MAS) may contribute to the success of breeding programs of *Varroa*-resistant lines of honey bees in the future, but, as stated by Rinderer et al. (2010), MAS will probably not be a guaranteed solution for making super-resistant bees. However, it may still become a useful tool for combining several resistance traits in the same stock. More than 10 years later, despite the steep growth of determined variant genotypes for *Varroa* resistance, MAS is still not commercially available in honey bees. The main reason is probably the genetic plasticity of resistance to *Varroa* (Traynor et al. 2020), which is also influenced by viruses and the environment. Research studies that identified QTLs, SNPs, and differentially expressed genes gave both exciting new evidence for complex genetic backgrounds as well as disappointingly inconsistent results between the studies (Le Conte et al. 2011; Navajas et al. 2008; Mondet et al. 2015; Behrens et al. 2011; Navajas et al. 2008; Mondet et al. 2015; Guarna et al. 2015; Parker et al. 2012; Gempe et al. 2012; Tsuruda et al. 2012; Arechavaleta-Velasco et al. 2012; Holloway et al. 2013; Spötter et al. 2016; Harpur et al. 2019; Conlon et al. 2019; Broeckx et al. 2019). However, Morfin et al. (2020) found that the expression of a gene associated with grooming behavior, AmNrx-1 (neurexin), was significantly higher in the selected stock (Indiana "mite-biter") than in colonies of unselected Italian bees. Guarna et al. (2015) researched proteins whose expression is tightly linked to hygienic behavior, Varroa-sensitive hygiene, and grooming behavior. They demonstrated a successful usage of an expression marker for selective breeding of disease-resistant stock. Those two studies are examples that are showing "firm" background of *Varroa* resistance could be found.

Thus, universal sets of markers that would enable MAS in the breeding of honey bees may be unrealistic, but the pinpointing of informative genetic markers in relatively closed and small honeybee populations is feasible and has been demonstrated as a successful approach (Bixby et al. 2017).

4. OUTLOOK ON POSSIBLE NEW APPROACHES FOR *VARROA* CONTROL IN THE FUTURE

Since *Varroa* will remain a major pest of honey bees in the coming decades, methods to reduce infestations will continue to be needed in addition to breeding efforts to select *Varroa*-resistant bees. The continuous search for or design of new acaricides for *Varroa* control is based on the mite's physiology and biochemistry (Dekeyser and Downer 1994) and on in silico screening (Riva et al. 2019) as well as its genomic sequence. However, many attempts were made in the field of biological control of *Varroa*. "Biological control is a component of an integrated pest management strategy. It is defined as the reduction of pest populations by natural enemies and typically involves an active human role." (Cornell University n.d.). Natural enemies include predators, parasitoids, and pathogens (e.g., bacteria, fungi, and viruses) and are referred to as biological control agents.

The different approaches to biological control had shown encouraging results when tested in the laboratory but still need further research and improvements for practical application in the field. Preparations containing such organisms must also pass an official approval process before they can be registered to be applied to food-producing animals. Further details about development of new acaricides and means of biological and biotechnical control as well as data-driven approaches for a more accurate *Varroa* management are presented in the table in Sect. 7.4 under Appendix.

4.1. Data-driven Varroa management

Successful *Varroa* control is strictly related to monitoring. Analytical software or artificial intelligence technologies could avoid mites to be counted by the beekeepers by analyzing images of bees or debris on bottom boards. An example of a software app to analyze images of live bees on frames is BeeScanning (https://beescanning.com/eng/; Michelsen 2018). The software counts the number of visible mites on bee's bodies and the number of bees, then it expresses the result as percentage. As the number of mites on top of bees' bodies is not the total number of phoretic mites—many of them are not visible in the images as they are hidden on the ventral side of bees' bodies—a factor is used to compensate the number of hidden *Varroa*. Another example of a tool to analyze images of *Varroa* is Apisfero's Bee *Varroa* Scanner (https://www.apisfero.org/). The device automatically identifies mites present on the sticky sheets placed in the bottom boards of the hives. It consists of five cameras mounted on a motorized slide, which allows to acquire high-resolution images of the sheet inserted in the device. The images are sent by the scanner to a cloud server, which processes them using an algorithm based on deep neural networks (deep learning) and distinguishes *Varroa* from debris particles and stains in the sheets.

The *Varroa* alert system (www.bienengesundheit.at), provided by the Austrian Beekeeping Federation, is one example of automated risk assessment tools (Morawetz et al. 2018). The web application analyses *Varroa* infestation data shared by beekeepers, who assess the *Varroa* infestation at least 3 times per year using a standardized protocol. A predictive algorithm extrapolates mite loads in individual colonies. Based on these predictions the system performs continuous risk assessments. In the absence of robust scientific data on the relationship between *Varroa* infestation at a given time of the year and the long-term colony survival, extension experts defined thresholds that are assumed to cause permanent damage to bee colonies if exceeded. These thresholds (natural mite fall of 3 mites day per day in May and 10 mites day per day in July), which are based on experience rather than empirical evidence, are fed into the predictive algorithm to estimate thresholds for every single week of the year. If the mean (median) of the measured (or extrapolated) infestation level of all colonies in each region for the current week is higher than 80% of the threshold of the current week, a " yellow" alert is triggered. If it is higher than 120% of the threshold of the current week, a "red" alert is triggered. Alerts are published online, via social media, mails alerts, and websites. Predictions can be customized to test the effect of treatments on mite infestation.

Based on a weather forecast data, the system also issues region-specific recommendations for an efficient application of veterinary medicinal products. Another approach, similar to the *Varroa* alert system, has been implemented by the Bee Informed Partnership (https://beeinformed.org) in the USA. This program, however, uses a different procedure to assess mite infestation: samples are processed by academic laboratory staff and additional information on hive health status and management are collected, allowing a more comprehensive analysis of the data. In contrast, researchers at the Center for Analytics Research & Education of the Appalachian State University in North Carolina (USA) are developing disease risk models based on data from apiary management software and automated remote sensing systems, allowing highly efficient real-time access to relevant information. But this is depending on a certain proportion of beekeepers deploying digital tools in their operations, which in turn depends on the structure and the economic capacity of the beekeeping sector in a certain region.

5. CONCLUSIONS

Honey bees are one of the most important managed pollinators worldwide, not only for many important crops but also for wild plants, thus ensuring food security for humans, livestock, and wild animals. *Varroa* (in combination with the viruses it transmits) will probably continue to be the main pest in managed colonies for the next decades in most parts of the world. It also poses a threat to feral honeybee populations. However, as these are not managed or selected to meet human husbandry objectives (e.g., strong and productive colonies, gentleness, low swarming tendency, transportability from crop to crop in pollination services), they mastered the challenge to develop resistance against the mite by natural selection and survived this way in some regions. The identification of *Varroa* resistance or at least resistance traits in some managed honeybee populations and consolidation by propagation of survivors or selective breeding for special traits (see Sect. 3.3) is an encouraging outlook for the future.

Simply using varroacides continuously leads to resistant mites but not to mite-resistant bees, as practice had shown in recent decades. Acaricide treatment also affects viral dynamics in *V. destructor*-infested honeybee colonies via both host physiology and mite control (Locke et al. 2012). According to Giuffre et al. (2019), the behavior of the mite is also influenced by the colony, DWV and SBV, respectively.

To maintain beekeepers' acceptance and to meet the needs of the beekeeping as well as the pollination dependent agricultural sectors, an integrated approach is a possible and feasible solution to keep the *Varroa* infestation below the treatment (economic) threshold or delay its onset (Delaplane et al. 2005), thus reducing the use of acaricides and reducing colony losses. It has to be noted that the goal of such an approach is neither the elimination of a pest or parasite from a honeybee colony nor the total elimination of treatments with varroacides, according to the definition by the FAO: "integrated pest management (IPM) means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment" (FAO).

The resources available in IPM to limit *Varroa* infestation can be considered a pyramid (Cornell University n.d.). The broad base is represented by the genetics of the host, followed in the mid part by apitechnical measures to reduce infestation. At the top is the application of acaricides if necessary, based on monitoring results and considering treatment (economic) thresholds.

Despite the encouraging examples of identified mite resistance in feral and managed colonies, it will take time to further select and establish this trait in the different managed populations of *A. mellifera* subspecies and breeding lines. IPM can contribute to bridging that time and to keep *Varroa*-associated colony losses at an acceptable rate to allow repopulation from surviving bees or local stock to meet the demands of beekeepers and growers of pollination depending crops.

The search for and development of new varroacides had changed considerably since the start of chemical *Varroa* control. Nowadays, a new and promising approach to search for new active ingredients for varroacides is the in silico chemical library screening, followed by experimental validation of identified possible novel compounds. By combining the in silico screening with in vitro experiments, two promising compounds (inhibitors of acetylcholinesterase in *Varroa*) were found, which are under further evaluation (Riva et al. 2019). Another encouraging approach was reported by Vu et al. (2020). They tested the voltage-gated chloride channel blocker DIDS. It showed a significantly greater field efficacy compared to Apistan® and CheckMite + ® against *Varroa* from hives for which tau-fluvalinate and coumaphos were ineffective. As these two examples show, research continues to develop and identify new acaricidal agents.

To delay or overcome the problem of resistance, the implementation of a resistance management plan for a region would be necessary, because mites carrying resistance genes are not restricted to single beekeeping operations but follow their natural routes of dispersion. Their presence will not be noticed by the beekeeper until he uses an active ingredient with existing resistance, thus giving insufficient mite control and increased colony losses (Lodesani 2004; Milani 2001). In conventional managed beekeeping operations, such a plan can include the coordinated annual rotation in the use of some acaricides with different modes of action (e.g., fluvalinate/flumethrin, amitraz, coumaphos). Since these substances are not allowed in organic beekeeping operations, effective alternative actives and application methods for *Varroa* control are urgently needed. This is also because biotechnical approaches (e.g., RNA interference, use of genetically engineered organisms) will probably not become an accepted method in organic beekeeping, at least not in Europe.

Apitechnical methods are rarely able to keep the mite infestation level permanently below the damage threshold without additional measures. Computer simulations indicate that non-chemical IPM practices delay damaging mite levels rather than prevent them (Hoopingarner 2001; Wilkinson et al. 2001).

Nevertheless, apitechnical methods are an important element in integrated *Varroa* control (Lodesani et al. 2014, 2019; Giacomelli et al. 2016; Gregorc et al. 2017). As demonstrated by Delaplane et al. (2005), apitechnical measures can be combined with selected *Varroa*-resistant bees. In their trial, the use of screen bottom boards and selected stock (hygienic or SMR) slowed down the mite population buildup and postponed the need for chemical treatments.

In an IPM approach, biosecurity measures in beekeeping (BMBs) are another important element. BMBs are defined as those integrated measures implemented to reduce the risk of the introduction and spread of specific honeybee disease agents (Pietropaoli et al. 2021). Well-implemented BMBs will avoid the introduction of new pests and pathogens and reduce pathogen loads. As a result, the use of veterinary medicines can be reduced, thus ensuring improvements in production quantity, quality, and safety (Dewulf et al. 2018). A prerequisite for the implementation of BMBs to beekeeping operations are good beekeeping practices (GBPs) defined as "integrative activities that beekeepers apply for on-apiary production to attain optimal health for humans, honey bees, and environment" (Rivera-Gomis et al. 2019). GBPs and BMBs must be implemented in beekeeping operations for the successful application and adaptation of the three pillars of *Varroa* management.

6. GENERAL SUMMARY

Varroa destructor is an ectoparasitic mite of two different *Apis* species (*A. cerana*, *A. mellifera*). It has spread almost all over the globe in the last decades of the twentieth century. By feeding on the fat body of the bees, it causes serious damage on the individual level and, by shortening the life span of bees, also on the colony level. Furthermore, it is a vector of viral diseases. The three pillars of *Varroa* management are the use of acaricides, the use of apitechnical measurements, and the breeding of resistant bees. In successful, sustainable *Varroa* management in good beekeeping practices the methods from all the three pillars that are used jointly and interchangeably.

Beekeepers often use preparations either called hard or soft acaricides for *Varroa* control. The so-called hard acaricides (e.g., coumaphos, amitraz, pyrethroids) are easy to use with high and constant efficacy if no resistant mites appear. In the case of resistant mites, the efficacy will decrease, and colony losses will increase.

Due to their fat solubility, residues may remain in bee products (mainly in beeswax and propolis, less in honey). If the wax residues exceed a certain level, they can migrate by diffusion from combs or foundation into the honey. Some other widely used acaricides, the so-called soft acaricides (e.g., formic acid, oxalic acid, and thymol) are known for their low risk of residues in bee products. But their efficacy varies among treated colonies and depends on environmental conditions. A resistance of mites to soft acaricides is not yet known and is believed to be rather unlikely. All the substances used for *Varroa* control must be approved by the competent authorities for the use in honey bees.

To overcome the disadvantages of both groups of acaricides (e.g., residues in hive products, resistance of mites, variable efficacy), an integrated approach in *Varroa* management is crucial. Examples of well-established techniques, so-called apitechnical control methods, are the removal of the drone and worker brood to eliminate a part of the mite population from the colony, brood interruption by queen caging to force mites to the phoretic stage, or the use of trapping combs to catch and remove mites. There are several promising methods that need to be further tested and improved in the future, including the use of bee- or *Varroa*-derived volatiles to trap the mites, the use of mite pathogenic fungi and bacteria, use of biotechnology, use of artificial intelligence, and the use of resistant honeybee stocks.

Bees exhibit three main behavioral characteristics related to *Varroa* resistance: hygienic behavior, which is defined as an uncapping and removal of diseased or damaged larvae; *Varroa* sensitive hygiene which is hygienic behavior, aimed specifically to detect, uncap, and remove *Varroa*-infested larvae; grooming behavior, which is the active removing of mites from the body of the bees. Suppressed mite reproduction—a recently observed and determined trait in certain honeybee colonies in which by different mechanism the reproduction of mites in cells is suppressed, leading to inability of *Varroa* infestation to spread.

There is still a lack of understanding of the genetic backgrounds of those behavioral traits due to huge genetic plasticity.

Beekeepers are the key factor for success or failure in *Varroa* management. They must be equipped with the knowledge in combining usage of different apicultural techniques and acaricides to successfully manage mites throughout the year and be skilled to detect and select for resistant honeybee lines.

AUTHOR CONTRIBUTION

JB prepared the first draft of the manuscript. All authors contributed equaly with suggestions, improvements and comments to the manuscript. All authors read and approved the final version of manuscript. Authors would like to thank anonymous reviewers and Katia Hinc for creating an image of three pillars in context of varroa control.

FUNDING

This work was prepared in the context of the B-PRACTICE project funding from the European Research Area on Sustainable Animal Production Systems (ERA-Net SusAn), co-funded under the European Union's Horizon 2020 Research and Innovation Programme (www.era-susan.eu) under Grant Agreement No. 696231. Funding parties on the national level are presented in the table below.

Country	Funding party	Funding program
Italy	Ministry of Health, The Directorate-General for Animal Health and Veterinary Medicines MoH DGSAF	Ricerca Corrente 2015
Austria	Bundesministerium für Land-und Forstwirtschaft, Umwelt und Wasserwirtschaft (BMLFUW)	PFEIL 15 (PFEIL15 (2010–2015) and followed by PFEIL20 (2016–2020)
Turkey	The scientific and technological research council of Turkey (Tubitak)	1001—Scientific and Technological Research Projects Funding Program
Slovenia	Ministry of Agriculture, Forestry and Food (MKGP) and Slovenian Research Agency/Javna agencija za raziskovalno dejavnost RS (own resources, 30%)	Kmetijsko znanstveno-raziskovalno delo and ARRS Research Program P4-0133 (own resources, 30%)

DECLARATIONS

Conflict of interest The authors declare no competing interests.

APPENDIX SELECTED METHODS FOR *VARROA* CONTROL AND PREVENTION OF VARROOSIS

Apitechnical measures

These measures are also applicable during periods of honey flow when any application of chemical treatments is omitted.

(Details are given in Sect. 3.1.)

Type of *Varroa* control	Activity	Mode of action	Application	Evaluation	References
Apitechnical measures (as sole measures or in combination with chemical treatments)	Trapping of mites in worker or drone brood	Natural host-finding behavior is used for trapping mites; mites in brood combs are killed by removal and melting, heat, or treatment	Total brood removal combined with trapping comb and classical trapping comb technique	Up to 95% efficacy in broodless colonies; up to 50% mites removable with solely drone brood removal; no detrimental effect on colony development; "resistance" of mites unlikely; method is applicable during periods of honey flow!	Beetsma et al. (1999); Calis et al. (1999); Charrière et al. (2003); Engels et al. (1984)

Type of *Varroa* control	Activity	Mode of action	Application	Evaluation	References
	Brood interruption	Creation of broodless conditions forces all mites to the phoretic stage, where they are accessible for chemical treatments or trap comb techniques	Queen caging, total brood removal combined with trapping comb and classical trapping comb technique, artificial swarms	Insufficient as a single method. Queen caging alone killed 40.6% of mites. In combination with organic acids, efficacy increased up to 97%. Trapping comb technique can reach an efficacy of 95% if it is done correctly (enough worker cells available to queen), possible losses of the queens	Giacomelli et al. (2016); Underwood et al. (2019); Büchler et al. (2020); Gregorc et al. (2017); Ribarits et al. (2020); Jack et al. (2020)
	Use of screened bottom boards	Mites that slip or drop accidentally or after behavioral defense of bees fall to the ground and are thus removed from the hive		Reduction of mite population from 13 to 21%	Ellis et al. (2001); Harbo and Harris (2004); Pettis and Shimanuki (1999); Ostiguy et al. (2000); Ostiguy (2000)

Type of *Varroa* control	Active agent	Mode of action	Application	Evaluation	References
Physical method	Heat	Application of heat to isolated brood combs without bees or to whole colonies kill mites on bees or within brood cells	Heat treatment with special equipment	Effective (especially on mites in treated brood combs) but costly on a time and material basis. Additional field trials needed to obtain more accurate information. Possible side effects on queen and drone fertility	Rosenkranz (1987); Bičik et al. (2016); Kablau et al. (2020); Wimmer (2015); Le Conte et al. (1990)

Chemical treatments with registered VMPs

In general, in most countries, chemicals used on honey bees against *Varroa* have to be approved and registered by the competent authorities so that they can be used legally in honeybee colonies. If specified, maximum residue limits in honey must also be observed, as well as respect for withdrawal periods after an application and mandatory use of personal safety equipment according to user manual.

In the EU, such chemicals are denominated as veterinary medicinal products (VMPs). As stated by law, only authorized active ingredients and registered products are allowed to be used on honey bees. The treatments listed below are restricted to the active ingredients used in AUTHORISED VETERINARY MEDICINAL PRODUCTS (according to CMDv/497311/2009 rev. 17 Amsterdam, 1 March 2021). A list of AUTHORISED VETERINARY MEDICINES FOR HONEY BEES IN EUROPE is available on the internet. See: https://www.hma.eu/fileadmin/dateien/Veterinary_medicines/CMDv_Website/Procedural_guidance/Miscellaneous/Bee_products_available_in_EU_2021.pdf (CMDv/497311/2009 rev. 17 Amsterdam, 1 March 2021)

A similar list is also available for the USA (EPA 2021).

As such a list is always subject to change according to changes in approved active ingredients or registered products it should be checked in every country before any chemical treatment against *Varroa* is planned or carried out. In some EU countries, there may also be some additional preparations registered according to national law (e.g., formic acid 60% *ad us vet*. or lactic acid 15% *ad us vet*. in Germany with a so-called "standard approval"), whereby they can be used legally.

Type of *Varroa* control	Active ingredients	Mode of action	Application	Evaluation	References
Chemical treatment with "hard" acaricides	Coumaphos	Coumaphos is an organophosphate, which acts as acetylcholinesterase inhibitor	Substances are applied via fumigation, trickling, spraying, and contact strips	Substances mostly lipophilic and persistent in wax; high risk to create residues in bee products (especially non-polar substances that are applied in strips); resistance of mites confirmed in many countries	Marchetti et al. (1984); Mehlhorn (2008, 2016); ; Bajuk et al. (2017); Bogdanov et al. (1998a and b); Johnson et al. (2009); Roth et al. (2020); Rosenkranz et al. (2010)
	Flumethrin Tau-fluvalinate	Flumethrin and Tau-fluvalinate are pyrethroids and act as open state voltage-gated sodium channel blocker		They are acting systemically or via contact	Berry et al. (2013)
	Amitraz	Amitraz interacts with octopamine receptors		Easy to use, effective, and economically efficient since they can be applied during the routine hive inspections. Hard acaricides can also negatively affect bees and brood	

Type of *Varroa* control	Active ingredients	Mode of action	Application	Evaluation	References
Chemical treatment with “soft” acaricides: organic acids	Formic acid*	Inhibits mitochondrial cytochrome c oxidase and acts by mitochondrial disruption and neurotoxicity	Applied in short- or long-term treatments in different applicator systems or as gel formulation. Different concentrations are used (60–80%), depending on registered product Acts as a fumigant	It is the only acaricide that is effective against phoretic and reproductive mites. Hydrophilic, therefore, no accumulation in beeswax. Trace amounts occur naturally in honey; contamination of bee products only if applied inappropriately; minimal danger of resistance of mites; efficacy (up to 97.2%) influenced by ambient temperature, concentration, hive size, amount of applied acid, placement, number of treatments and colony strength; high concentrations harm brood and can cause queen losses; personal safety precautions for the user necessary; recently developed gel formulations facilitate application, but also can cause brood or bee damage *Legalized in Germany by a so-called “standard registration”	Bolli et al. (1993); Fries (1989); Hoppe et al. (1989); Satta et al. (2005); Elzen et al. (2004); Song et al. (2008, 2009); Giusti et al. (2017); Gregorc et al. (2016)
	Oxalic acid/ oxalic acid dihydrate	Exact mode of action is unknown, acts probably on mitochondrial function due to the effects of low pH and strong acidity of solution	Applied by spraying, trickling, or sublimation, according to the registered products. Acts by direct contact	Efficacy > 90% when colonies are broodless; less than 60% in colonies with brood; efficacy independent from temperature; negative effects on brood and bees when repeatedly applied in short intervals on the same bee generation. Application via sublimation gives higher mite mortality (97.6%) and lower bee mortality. Resistance is less likely	Bacandritsos et al. (2007); Charrière et al. (2002); Gregorc and Planinc (2001); Higes et al. (1999); Nanetti et al. (1997); Al Toufailia et al. (2015); Johnson et al. (2010); Wallace et al. (1997); Jack et al. (2020, 2021)

Type of *Varroa* control	Active ingredients	Mode of action	Application	Evaluation	References
	Lactic acid*	Mode of action is not known, supposed to be due to the low pH, thus interfering with the metabolism of the mite	Spraying comb by comb of a 15% aqueous solution in broodless colonies onto bees on each side of the comb. Acts by direct contact	Application during the broodless winter period at low environmental temperatures (4–10 °C) or in summer after brood interruption or onto swarms Efficacy up to 96% (three applications in winter) but not constant and frequently insufficient *Legalized in Germany by a so called "standard registration"	Koeniger et al. (1983); Assmann-Werthmüller et al. (1989); Rosenkranz et al. (2010) in Roth et al. (2020); Weiss (1987, 1991)
	Hop beta acids**	It is supposed to cause death by asphyxiation by penetration of the pest's thin exoskeleton Weak organic acids; non-toxic	Strip formulation which has to be placed in the brood chamber to give maximum efficacy. Acts by direct contact	**USA: a product of potassium salts of hop beta acids (K-HBAs) registered EU: not approved and not listed in the database of authorized bee products	EPA (2015a; b) CMDv (2021); Rademacher et al. (2015); DeGrandi-Hoffman et al. (2012); Rademacher et al. (2015)
Type of *Varroa* control	**Active ingredients**	**Mode of action**	**Application**	**Evaluation**	**References**
Chemical treatment with "soft" acaricides: essential oils	Thymol Blend of thymol, camphor, menthol, eucalyptus oil	Thymol: acts on the octopaminergic system, tyramine, and GABA receptors Menthol acts as a GABA receptor modulator. as an analgesic, mediated through selective activation of κ-opioid receptors and blocks voltage-sensitive sodium channels Camphor acts as central nervous system stimulant; is an antagonist of nicotinic acetylcholine receptors (nAChRs) and is neurotoxic Eucalyptol: It is described to have anti-feedent, repellent, ovicidal, larvicidal, pupicidal, and adulticidal effects	Applied as gel or solid carrier-based formulation on top bars of the brood combs	Thymol-based preparations should be applied only in case of low *Varroa* infestation level. Treat all colonies in an apiary at the same time to prevent robbing. Treatments during honey flow will give a thymol smell to the honey. Resistance less likely	Lindberg et al. (2000); Gregorc et al. (1996); Mattila et al. (2000); Bogdanov (1998); Chengala et al. (2017); Klocke et al. (1987); Sfara et al. (2009); Enan (2005a, b)

Selective honeybee breeding for resistance to *Varroa*

Type of *Varroa* control	Mode of action	Application	Evaluation	References
Selective honeybee breeding	Several mechanisms: hygienic behavior (HB), *Varroa* sensitive hygienic behavior (VSH) and suppressed mite reproduction (SMR), grooming behavior (GB, postcapping stage duration (PSD), recapping (REC), reduced mite population development (MPD)	Search for survivors in feral and managed populations, respectively. Requeening with resistant stock. Colonies that exhibit significantly lower *Varroa* infestations have higher survival rates when infested or exhibit desired behavioral traits, which are suitable for further breeding. *Varroa*-sensitive hygienic (VSH) bees: Minnesota hygienic line, grooming behavior bees, ankle-biter bees	In order to include selection for *Varroa* resistance successfully into breeding programs, close cooperation between all stakeholders within and around the breeding program is necessary. The interest in and preference of beekeepers for this trait as part of a breeding goal is important and should be considered, as well as proper management to assure successful collective selection to enable the sustainability of such a breeding program	Uzunov et al. (2017); Guichard et al. (2019); Locke (2016), Oddie et al. (2017), Guichard et al. (2020); Le Conte et al. (2020), Mondet et al. (2020); Spivak et al. (2021), Büchler et al. (2010); Guarna et al. (2015); Morfin et al. (2020)

Outlook on possible new approaches for *Varroa* control in the future

In Sect. 4, different approaches of possible methods for *Varroa* control in the future are listed. All of them are under research and evaluation for relevance. At this time, they all are in an experimental stage and much research still must be done to prepare for the next steps (e.g., evaluation of efficacy; risk profiles for humans, honey bees, bee products, and the environment) before they can be submitted for approval of the actives and registration of a product. Therefore, some time will pass before they are applicable in beekeeping practice and will help to mitigate the *Varroa* problem.

Type of *Varroa* control	Activity	Mode of action	Application	Evaluation	Reference
New acaricides	Search for and development of new actives	Lithium chloride: systemic mode of action	Feeding of a solution	It was effective against the mite but highly toxic for the honeybee brood. Reported residues in larvae, honey, and bee bread after test applications of lithium chloride To date, it has not been approved to be used on honey bees and may have serious negative consequences for humans if honeybee product containing the lithium chloride is ingested	Ziegelmann et al. (2018); Boecking et al. (2018); Prešern et al. (2020)
		Synthetic analog of costic acid; mode of action unknown	By contact (laboratory test)	This sesquiterpene-carboxylic acid, present in the plant *Dittrichia viscosa* showed an acaricidal effect on *Varroa* in the laboratory	Georgiladaki et al. (2020)
Use of chemical ecology	Use of bee-derived volatiles	Confusion of host-finding behavior of *Var-roa* by evaporation of synthetic volatiles, which interfere with the process of cell invasion	Strips	Field tests missing; inconsistent results of laboratory assays (particularly e.g., fatty acid esters). Deterrent activity of royal jelly; efficacy in the field still unclear	Pernal et al. (2005); Ding (2010); Niu (2014)
	Use of *Varroa*-derived volatiles	Reduction of the reproductive success of the mite	To be developed	This reduced copulatory activity in young females but would not completely stop the growth of a *Varroa* population; improvement of the application technique is still needed	Ziegelmann et al. (2014)

Type of *Varroa* control	Activity	Mode of action	Application	Evaluation	Reference
Biological methods	Use of pathogenic fungi	Pathogens cause lethal infections on mites	Application of conidia (asexual spores on bees/in-hive by spraying, trickling, or suspending impregnated strips)	*V. destructor* has been reported to be susceptible to the entomopath-ogenic fungi *Metarhizium. anisopliae*, *Verticillium lecanii*, *Hirsutella thompsonii*, *Beauveria. bassiana.* Contradictory reports regarding impact on mites and bees/brood are published. High temperature in hive is also a problem for fungal growth. However, when spores of *B. bassiana* were sprayed inside hives, adult bee mortality did not differ from control treatments	Fernandez-Ferrari et al. (2020); Shaw et al. (2002); Hamiduzzaman et al. (2012)
	Use of pathogenic bacteria	Pathogens cause lethal infections on mites	To be developed	The best results were obtained with isolate EA49.1 (*Bacillus thuringiensis*), which yielded 100% mite mortality. Field trails are still needed In another approach in a laboratory bioassay, mites were sprayed with the spent medium of 6-day-grown bacterial cultures. Strains of *Lactobacillus kunkeei*, *Bacillus thuringiensis*, and *Bifidobacterium asteroids* caused 95–100% mortality of mites in 3 days, indicating a miticidal effect of unidentified mode of action	Tsagou et al. (2004); Alquisira-Ramirez et al. (2014); Saccà and Lodesani (2020)

Type of *Varroa* control	Activity	Mode of action	Application	Evaluation	Reference
	Use of a benign/less virulent haplotype of *Varroa*	Reproduction of mites is influenced by competition for resources in multiply infested cells. In theory, establishing a benign/less virulent population of *Varroa* by inoculation/introduction could therefore induce reproductive suppression in the virulent type		This theoretical approach has already become obsolete by contrary evidence from Brazil. There, the original Japan/Thailand haplotype has been replaced by the more virulent Korean haplotype and a corresponding increase in both the mite fertility (from 35 to 72%) and the number of mites producing at least one viable offspring in worker brood (from 56 to 80% In addition, in Thailand, microsatellite marker-based evidence suggested hybridization between *V. destructor* and *V. jacobsoni* in single honeybee colonies infested with both mite species	Vetharaniam and Barlow (2006) Carneiro et al. (2007) Dietemann et al. (2019)
Biotechnological methods	Use of RNAi technology	Double-stranded RNA applicated per os to bees in sugar syrup is transferred to the mite and interferes with mRNA of the mite for *Varroa*-specific proteins ("gene silencing")	Per os application in sugar syrup	Efficacy up to 61%, bidirectional horizontal transfer between bees and mites, dsRNA degrades in 6 days in hive conditions. No effect on bees. Selected sequences are not homologous to honey bee or human sequences	Garbian et al. (2012), Niu et al. (2014); Ding et al. (2010); Huang et al. (2019)
Data-driven *Varroa* management	Use of artificial intelligence	Hardware or forms which are filled in by beekeepers for data collection and software-based locally or in cloud servers (algorithms based on deep neural networks and other modeling methods)	Many different sensors, like cameras, scales	Can offer good tools for beekeepers to plan interventions against mites and have great potential to predict certain scenarios based on recordings. However, many of them are relying on data collected by beekeepers (manually)	Michelsen (2018); Morawetz et al. (2018); https://beescanning.com/eng/; https://www.apisfero.org/; www.bienengesundheit.at https://beeinformed.org

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