# Control, Biocontrol and IPM Perspectives of Varroa Destructor

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Varroa destructor is a parasitic organism feeding and living among honeybees. It transmits viruses like the Deformed Wing Virus which can lead to the decline and death of the colony. Many treatments have been developed over the years like formamidine amitraz, pyrethroid tau-fluvalinate, organophosphate coumaphos or even acids like formic and oxalic to control the spread of the mite. However, none of this solution provides long-term sustainability for honeybees and no resistance from V. destructor. Therefore, the development of alternative tools remains open.

Keywords: Varroa Destructor ; honeybees ; integrated pest management ; biocontrol ; holistic approach

## 1. Introduction

While honeybees forage, nurse, reproduce, eat, or communicate in an already critical unhealthy environment with pesticides [1], climate change [2] or habitat loss [3] , parasites take their chance too [4]. One of them is Varroa destructor , a world major threat against bees [5][6]. After a shift from its original host the Asian bee Apis cerana to the Western honeybee Apis mellifera , it rapidly spread in the 1970s in Europe and in the 1980s in America [5]. It is now observed in both managed and wild A. mellifera [7]. Due to a shorter coevolution time between A. mellifera and V. destructor [8] as well as fundamental biological differences, the Western honeybee is far more impacted by the mite than the original host [5][9][10][11].

Why is this ecto-parasite such a threat for A. mellifera worldwide? It appears that V. destructor is tightly connected to several viruses and especially the DWV (Deformed Wing Virus) with its diverse variants DWV-A, DWV-B originally known as VDV-1 and DWV-C [12][13][14]. This RNA virus is responsible for wing malformations in bees, causing flight incapacities, thus a lack in food collection for the colony but also a threat to pollination [15][16]. Assuming the transmission of the virus occurs at the adult stage, no visible symptoms are reported [17] but a shortened lifespan is described [18]. The infection at the larval stage (emphasised through V. destructor ) induces damage like shortened abdomens, reduced brood nursing and learning deficits [19][20]. From a host-parasite perspective, interactions have to be considered not as a duo but as a triangle, highlighting the virus quasispecies which can spread between bees and acari [17][21][22][23][24][25][26].

Fifty years of intense research about V. destructor support an impressive amount of knowledge in order to deal with practical issues: how to reduce the impact of the mite on honeybee populations [27]? It is true that current control methods efficiency for the acari are still debated. Hard chemicals like pyrethroids, formamidine, organophosphate, neonicotinoid, or sulfoximines were or are still used in the field. However, their negative impact on honeybees' cognition is now widely identified and the resistance developed by the mite is part of current knowledge [28][29]. Other solutions were explored too, like drone removal, brood interruption, or breeding programs [30][31]. Soft acaricides such as thymol, hop leaves or acids seem promising for some [32][33] and already trouble making for others [34][35].

As urged by the integrated pest management program (IPM), a more integrative view is compulsory. The goal is not anymore to kill each and every one of them but rather to reduce their impact without harming honeybees and other wild species around. New strategies based on IPM and biocontrol are passionately studied all over the world. Currently, none is adequate to reduce the adverse impact of mites on bees. It is imperative to develop a holistic approach that focuses on the complete understanding of V. destructor biology and its tight relationship with its host. Health biomarkers should be determined for the mite and would help to evaluate at sub-lethal level on a long-term period the impact of molecules or biotechnics. This integrative approach involves in silico, in vivo, semi-field and field scales. Our review aims to discuss the latest ideas about control, IPM and biocontrol for A. mellifera against V. destructor from the laboratory to the field in realistic reproductible and applicable conditions.

## 2. Know Your Challenger, Varroa destructor

ORIENTATION— Many olfactory signals from adults, brood, or colony matrices have elicited behavioural responses from the mite. For instance, specific blends of fatty acid esters from old bee larvae [36][37] or aliphatic alcohols and aldehydes from cocoons [38] were shown to trigger the arrest or even the attraction of female acari in laboratory conditions. Brood food holds 2-hydroxyhexanoic acid, a volatile blend which also appeals dispersive mites [39][40]. Conversely, the mite is blocked by the ω-functionalised fatty acids from royal jelly [41] and the larger amount of methyl oleate in royal cells [42], preventing the parasitisation of queen brood. The ecto-parasite is also blocked and pushed away by two components of the Nasonov pheromone, geraniol and nerolic acid [43] as well as (Z)-8-heptadecene [44]. It turns out that foragers emit more of this semiochemicals and the (Z)-8-heptadecene than nurses. Therefore, it partially explains why dispersal mites are able to choose nurses over foragers [45][46].

REPRODUCTION— In the dark environment of the capped cell, the male acari has to recognise and mate specifically with the mature unmated females. The female actually emits a volatile sexual pheromone, composed of oleic acid, palmitic acid, stearic acid and their ethyl esters which attracts the male through its tarsal sensory pits and triggers its courtship behaviour [47][48]. The youngest daughter seems to be always the favourite choice of the male because the emission of the sexual pheromone is stronger in young and reduced in older females [47]. In the same way, once the mite is inside a capped brood cell, it is the shift in the fatty acid ester compound produced by the bee pupa (a decrease of ethyl esters and an increase of methyl esters) that initiates the reproduction and egg laying through vitellogenin induction [49][50]. Even the sex determination seems to be driven by bee pheromones as Garrido and Rosenkrank [51] showed. In that case, fatty acid esters volatile signal triggers a male egg laying by the foundress. Supporting the hypothesis of a complex chemical environment ruling V. destructor behaviour, Frey et al. [50] demonstrated that artificially inserted mites with methyl esters compound stop reproduction. Plettner et al. [39] hypothesised that this signal alone could indicate to the mite that the bee pupa development is too advanced for the offspring to reach adulthood before the honeybee emergence.

According to the period and the hive environment, the behaviour of V. destructor is quite different and extremely adaptive [52]. At low mites abundance in the colony, acari seem to prefer nurse bees over foragers or new born bees [53], based on differential pheromonal signatures between nurses and foragers. Yet, the mite can passively modify the hydrocarbon cuticle of its host according to colony infestation levels. At high infestation levels, nurses and foragers, less discernible by their different cuticular hydrocarbons, are equally appealing to V. destructor , thus promoting the exploration of new bee colonies [54].

This natural drifting of mites between colonies is one of the factors which increases the deleterious effect of the ecto-parasite on bee populations, especially through viral transmission. The biology of the ecto-parasite is indeed not complete without the study of its viruses like the Deformed Wing Virus.

## 3. Varroa destructor Chemical and Semi-Chemical Control Methods

The off-target effects, along with the increasing resistances of mites, pushed for new ways of pest control [55] like soft acaricides.

Many molecules from natural origins were considered as alternatives to synthetic chemical treatments. Thymol, formic acid or oxalic acid have been stressed since the 1970s–1980s as efficient treatments against the ecto-parasite [56]. Yet, the ‘perfect’ solution does not exist, and new molecules are tested each year in the hope to come up with a long-term answer. Some of these molecules have been used for decades without a clear understanding of their mode of actions on both the honeybee and the acari. This is for instance the case of formic acid.

FORMIC ACID— Formic acid holds a great potential since it is the only molecule, so far, able to reach both dispersal and reproductive mites inside brood cells [57]. When used as a treatment, formic acid seems to interfere with the cellular respiratory chain, more precisely the cytochrome C oxidase. It inhibits the oxidative phosphorylation, thus impacting the mitochondrial energy metabolism [58]. Genath et al. , (2020) [59] studied the transcriptome of A. mellifera and V. destructor after topical formic acid treatment and highlighted a difference in detoxification capacity between the host and the acari. Their work supports the hypothesis of interference with cellular respiration through the modified expression of several genes like cytochrome P450 suggesting a stronger toxic selectivity toward the mite. To date, no resistance was detected in V. destructor [39][48]. Hansen et Guldborg (1988) [60] showed that the formic acid concentration increasing in honey after a treatment was not sufficient to be harmful and persistent in time [61][62]. Nevertheless, many parameters such as the delivery methods, the size of the hive, the position of the evaporator in the hive, the humidity and the temperature are known to greatly affect the treatment efficacy [63][64][65][66]. For example, high temperature combined with low ventilation in-hive may lead to higher brood toxicity and lower mite mortality due to quick evaporation rates [67]. In addition, several studies described some drawbacks like swarming, queen mortality, damaged young bees or reduction of sealed brood [65][68][69]. At sub-lethal doses, formic acid involved memory impairment for bees in the short and long term [70]. Still, this acid seems a good compromise to keep a reduced number of mites without drastic honeybees loss [65]. In addition to health risk for the user in case of incorrect use, real efficacy is known to variate throughout the world, from 39.8% of mite mortality in the USA [67] to 92% in Argentina [71].

OXALIC ACID— Oxalic acid is a natural acaricide in use since the 1980s against the mite. Again, this acid is a molecule naturally present in honey [61]. Due to its hydrophilic nature, oxalic acid is used as an acaricide treatment and does not lead to high residual concentration in wax [72][73][74][75]. It kills dispersive mites on honeybee body but cannot penetrate a brood cell, limiting its effects [73][76][77]. In field trials, Maggi et al., (2016) for instance, showed a miticide efficacy of 93.1%. Surprisingly its mode of action towards V. destructor is still unclear although it is most likely mechanical [48]. Sublimation method seems to cause crystallisation of the acid on the acari's body, leading to the inability of the mite to adhere to any substrate [78]. The fact that V. destructor appears unable to detect this acid by olfaction [78] and the putative mechanical mode of action could reduce the chance of resistance from the mite [48]. No resistance was observed over 8 years of treatment in a recent bioassay [32]. Yet, caution should be taken as bacteria characterised from V. destructor microbiota were shown to express oxalotrophy. This gives them the ability to degrade oxalic acid in order to use it as their carbon source [79], thus conferring resistances to the carrying mites. Despite its use as an organic treatment, oxalic acid at high and sub-lethal doses can still be harmful to the bees. Severe and irreversible internal tissue damages [80][81] or disruption of the proteolytic activity of the cuticle were observed, impeding bees' immunity [82]. Administration method is actually a key point and higher death rates were associated with oral exposure when compared to topical application [83]. Maggi et al. [32] suggested that the combination of glycerol with oxalic acid may prevent honeybees from oral ingestion, reducing deleterious effects, without reducing the efficacy of the acaricide treatment. Besides the effect on adult bees, experiments led on larval stages with spray application showed midgut damages as well [84]. Finally, tests on long-term effects on colonies characterised loss of brood, workers and sometimes queen according to the concentration used [73][85].

## 4. Biocontrol and IPM Strategies for V. destructor Management

Here we summarised the main roads of IPM and biocontrol for honeybees against the ecto-parasite explored by scientists.

Often combined with organic molecules, a mechanical approach can be an alternative as well. The main goal is to perform total brood interruption, removal of drone brood, queen caging or trapping combs to decrease the pressure of V. destructor population on the colony [74][86][87]. In fact, these mechanical methods allow to artificially create a broodless period where mites have to be on adult honeybees, making them accessible to molecules. The removal of brood frames after a broodless period can also allow the trapping of an important number of reproductive mites. Unfortunately, according to the region of the world, brood interruption does not give the same results. It was the best solution to lower the ecto-parasite pressure on colonies in several countries in Europe but not in the USA where it affected their strength and survival [87][88][89][90][91]. Another method called sugar shake, used as a diagnosis method, reduces as well the number of mites on adult honeybees and lower the pressure on the colony without causing deep damages [92].

Second, the predatory mite Stratiolaelaps scimitus is another potential candidate studied for biocontrol solution. Despite an effective ability to kill V. destructor , they also prove to prefer the eggs of the honeybee to the mite [93]. Moreover, in field experiments, an introduction in early and late fall did not lead to a decrease in the acari pressure upon colonies [94][95].

Rather than targeting the mite, honeybee microbiota can also be used to improve health and resistance in the host. Probiotics were already considered to enhance the immune system against other threats to honeybees like American foulbrood or Nosema ceranae [96][97][98]. As the acari was spotted to disturb honeybees' gut microbiota (dysbiosis), it appeared that probiotics, like Gram-positive bacteria Lactobacillus and Bacillus strains, brought beneficial impacts on colony health and seemed to reduce the incidence of the mite [99][100][101]. Bacteria communities appealed as well for their released metabolites. They were tested as treatment against the ecto-parasite. Lactic acid from L. johnsonii AJ5 induced the mite's death when fed to bees. The mechanism implied in V. destructor 's mortality remains unknown and needs further confirmations [102][103].

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