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RECENT DISCOVERIES IN *Varroa destructor* TREATMENT, PREVENTION AND PARASITE - HOST INTERACTION

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Abstract

The European honey bee, Apis mellifera, as most insects of the world, is currently facing major difficulties and, particularly for honey bees, this results in significant colony losses. One of the most stressful factors for A. mellifera is the ectoparasitic mite, Varroa destructor. V. destructor invasions are largely treatable and preventable, however they bring forth great challenges to A. mellifera populations and breeders, making apiculture increasingly time and resource consuming. The global research in apiculture pathology is mostly focused on Varroa sp. This review will be focusing on the recent literature in Varroa treatment, prevention and parasite - host interaction.

Key words: *Apis mellifera, Varroa destructor, pathology, host-parasite interaction.*

INTRODUCTION

Varroa destructor's original host was the Asian honeybee, *Apis cerana* which through coevolution was able to develop tolerance toward the mite. This trait was not carried over to the western honeybee, as the host-parasite relationship between *Apis mellifera* and *V. destructor* is relatively recent (Le Conte et al., 2007). A *Varroa* infestation can, therefore, eradicate a colony of *A. mellifera* within 1-3 years, if left untreated. The lack of a balanced host-parasite relationship between the European honeybee and the mite has facilitated a world-wide spread of *Varroa*, within a relatively short period of time.

A codependent relationship with humans means they always receive adequate treatment against infestations, in order to keep the colonies healthy and productive. At first glance, this relationship may seem advantageous for the bees, because they don't have to suffer major losses or be weakened by *Varroa* infestations, however at a second glance, such grooming hides a darker side. Namely, it breaks the cycle of natural selection which is required to become tolerant to pathogens. Thus, under constant treatment, any individual who can reproduce, regardless of its genetic sensitivity, is able to pass on its genes, which hinders the possibility of host adaptability. Examples of *V. destructor* resistant *A. mellifera* populations can be found in most parts of the world (DeJong et al., 1997; Fries et al., 2006; Le Conte, 2007). These honey bee populations prove that through long-term exposure to the *Varroa* mite, resistance can be developed.

This review will focus on recent discoveries in the host-parasite relationship of *Varroa destructor* and *Apis mellifera*, new treatment methods and the underlying mechanisms of resistance towards the mite.

Varroa destructor AND VIRAL INFECTIONS

In addition to the numerous negative effects *Varroa* directly inflicts upon *A. mellifera*, mite infestations are usually also associated with viral infections (reviewed by Tantillo et al., 2015). Recent research has helped shed light on *Varroa*'s role as a viral vector and how infections can become a contributing factor in colony losses.

Deformed wing virus (DWV) copy number in honeybee pupae is directly associated with the copy number in infesting *V. destructor* (Wu et al., 2017). The presence of large DWV copies induces immunosuppession in the honeybee in order to more easily replicate (Di Prisco et al., 2016), which acts as an additional stressor and adds to the likelihood of a colony to perish.

Studies suggest that a longer phoretic stage does not necessarily mean a more successful reproductive cycle but that the longer the phoretic stage lasts, there is a higher chance of deformities to appear on the young honeybee. Additionally, DWV load increases with the time spent in the phoretic stage, thus leading to more frequent and severe infections (Piou et al, 2016).

DWV severity, transmitted by *V. destructor* can be dependent on the climate. Overt infections are much more common in temperate climates than they are in tropical climates, without any differences in infestation rates (Anguiano-Baez et al, 2016). This could happen in part because *Varroa* is a better vector for viruses in temperate climates. This theory is supported by Giacobino et al. (2016), who showed that colonies in temperate climates had a much higher viral load compared to colonies in subtropical climates.

This study, however, also reports that *Varroa* infestation levels were higher in temperate climate compared to tropical climate, as was the case for viral load. Currently there is no knowledge of DWV in honeybees in Australia (Roberts et al., 2017). This could be due in part because *Varroa destructor* has only recently been able to spread to this continent and because Australia's climate is partly tropical and mostly arid, which, as established above, are poor conditions for the DWV. The fact that *V. destructor* infestations are milder in Australia and usually doesn't lead to colony losses supports the idea that honeybee mortality is a result of multiple stress factors working together against the bees.

FRESH INSIGHTS IN METHODS OF *Varroa* CONTROL

As far as *Varroa* control goes, the most efficient and widely used methods consist of either synthetic 'hard' chemicals or plant based 'soft' chemicals (Rosenkranz et al., 2010). These treatments function as miticides against *Varroa* and, although effective, they also bring numerous negative side effects for the honeybees, including mortality (Gregorc et al., 2018). Severity of these effects is dependent on the age of the bees and on the level of social interaction (Van Buren et al., 1992). An additional disadvantage to chemical treatments is that *Varroa* can become resistant, which is why efficient management practices are equally as important in *Varroa* control (Thoms et al., 2018). Environmental conditions seem to be the predominant factor in mite infestation levels, followed closely by beekeeper management (Giacobino et al., 2017).

No new active compounds against *Varroa* were discovered in the past 25 years (Mutinelli, 2016), although some recent studies present promising results. Lithium salts were shown to completely eliminate *Varroa* mites in caged environments, without affecting worker bee mortality as compared to untreated controls (Ziegelmann et al., 2018).

Plant extracts offer a great alternative to conventional chemical treatments. These "soft" chemicals offer a similar antiacaricidal effect and are potentially less toxic.

Fumigation with oregano essential oil can rid a colony of *Varroa* within the first two weeks of treatment, while not showing toxic effects towards the honey bees. The constant output of essential oil through fumigation results in a more efficient treatment (Sabahi et al., 2017). Plant based extracts such as *Thymus algeriensis* essential oil also offer a great solution against *Varroa*.

This extract contains large quantities of thymol, which is a known antivarroa agent (Noureddine et al., 2016) and has been shown to reduce mite populations by 32.6%, without harming the honeybees (Kouache et al., 2017). Mild acaricide effect was shown in sage - *Salvia officinalis* L. (Lamiaceae) - essential oil (Bendifallah et al., 2018) and costic acid extracted from *Dittrichia viscosa* proved to be 80% as effective as commercial acaricides (Sofou, 2017).

In addition to good management practices and chemical treatments, the use of technological methods, like the removal of drone brood (Wantuch and Tarpy, 2009) offers an efficient and cost effective solution against *Varroa*. Irradiation of honeybee colonies did not seem to influence *Varroa* infestation levels and overall effectiveness in pest control could be described as mild, at best (de Guzman et al., 2019).

The use of *Stratiolaelaps scimitus*, a mite that feeds on small insects, showed promising effects against varroa infestations. This method isn't 100% safe though, since the mite also consumed honeybee eggs in lab conditions, but not in the hive (Rondeau et al., 2018) and treatment applied in late or early fall was not efficient in controlling varroa (Rondeau et al., 2019).

*Bacillus thuringiensis*is is virulent and pathogenic in small insects and acarids, including varroa (reviewed by Chandler et al., 2001), however, it does not affect the honeybee and can be used alongside conventional treatments for *Varroa* control (Alquisira-Ramírez et al., 2017). Overall, bacteria, especially from the *Bacillus* and *Lactobacillus* genus, act as probiotics and bring important benefits for the honeybees by increasing the immune response, stimulating queen egg laying and significantly increasing honey yield (reviewed by Audisio, 2017)

Entomopathogenic fungi could also reduce varroa damage to honeybee brood by both infecting the parasite and preventing varroa-associated suppression of honeybee immunity. Three immune genes of the honeybee, hymenoptaecin, pUf68 and BlCh, are usually suppressed by varroa. When inoculated with *Metarhizium anisopliae* and *Beauveria bassiana*, varroa cannot suppress the expression of these genes (Hamiduzzaman et al., 2012).

The sensory limitations of the varroa mite can be used against it. Given the lack of sight, the varroa mite is dependent on chemoreceptors to find its next host (Dillier et al., 2006). By inhibiting the chemoreceptors, varroa will have difficulties in choosing the right host. One way in which olfactory detection can be inhibited is through the use of racemic compounds (Govardhana et al., 2016)

In addition to grooming and hygienic behaviors, honeybees were also found to change normal behavior in order to alleviate pathogenic pressures. *A. mellifera* colonies have been found to change foraging patterns as a response to pressure from varoosis. Colonies infested with *V. destructor* increased the number resin foragers, thus increasing the quantity of collected resin as a means of self-medication (Pusceddu et al., 2019).

TREATMENT RESISTANT MITES

Chemical treatments offer the most effective solution for treating varoosis but they also bring forth multiple downsides amongst which toxicity for the honeybees, pollution of bee products and development of treatment resistance in *Varroa* (Rosenkranz et al., 2010). While product pollution and toxicity are negligible in terms of severity and economic impact, the spreading of treatment resistant *Varroa* mites could be disastrous for honeybee populations. The following part of the review will be focusing on recent scientific discoveries in resistance to treatment.

Evidence for resistant *Varroa* populations has started to emerge at the end of the 20th century (Lodesani et al., 1995; Hillesheim et al., 1996; Milani, 1999) and continue to emerge to this day. Recent studies have helped shed light on resistance mechanisms. A link was found between two novel mutations at Leucine 925 of the Voltage-Gated Sodium Channel gene (L925M, L925I) and resistance to pyrethroids, tau-fluvalinate and flumethrin, in USA (Gonzales-Cabrera et al., 2016). Mutations at this residue were also found in Pyrethroid resistant mites from Southern England (Gonzales-Cabrera et al., 2013) and in the Czech Republic (Stara et al., 2018; Hubert et al., 2014). This mutation was found in 98% of mites that went through miticide treatment and in only 45% of non-treated individuals which means that when selective pressure is applied, mite populations can develop resistance to the treatment. A connection between point mutations at position 925 in the sodium channel gene and treatment resistance has been confirmed in a biological assay (Stara et al., 2019).

Varroa destructor is a highly inbred species, due to its reproductive mechanism. Genetic diversification only occurs once the *Varroa* population grows, in the middle of the honeybee productive period, when brood cells are populated by more than one foundress. Applying antivarroa treatments before this stage, when the *Varroa* population is low and goes through a population "bottleneck" could help with fixating variants responsible for miticide resistance (Beaurepaire et al., 2017).

These findings are alarming considering the slow development of new control methods and the fast spreading of the mite. Hierarchical genetic variation can be found at a colony level, which indicates that *Varroa* transmission doesn't only happen vertically from one generation to the next but also horizontally, between hives and apiaries (Dynes et al., 2016). Horizontal transmission is facilitated by *Varroa*'s capacity to quickly climb on its host (Peck et al., 2016). *V. destructor* has also been found in drone congregation areas, which increases the mite's transmission capabilities even further (Mortensen et al., 2018).

Luckily, though, bee populations have a few aces up their sleeves.

WESTERN HONEYBEE RESISTANCE AGAINST *Varroa*

The oldest Western honeybee population resistant to *Varroa* was recorded in 1997 by De Jong and coworkers. Twenty Italian honeybee colonies infested with *Varroa* were brought in 1984 to the Island of Fernando de Noronha, off the coast of Brazil. They were genetically isolated, as to prevent genetic contamination and were left to face *Varroa* without any treatment. This population survived the infestation and is still alive to this day (De Mattos et al., 2016).

The first experimental insight on *A. mellifera* resistance to *V. destructor* was brought forth in 2006 by Fries et al. After three years a *V. destructor* infested, untreated *A. mellifera* population of 150 colonies had an 80% mortality rate during winter. This rate decreased to 13% and 19% in the 5th and 6th year respectively, while infestation levels in the fall also significantly decreased. This is a great example of adaptability by *A. mellifera* and *V. destructor*, and proves that coevolution is possible when selective pressure is applied.

Varroa surviving colonies also show a similar mortality rate when compared to treated colonies, at the expense of lower honey productivity (Le Conte et al., 2007).

When compared to control populations, *V. destructor* resistant colonies have a similar hygienic and grooming behavior but the reproductive success of *Varroa* is significantly reduced (Locke and Fries, 2011). When compared to *A. mellifera,* mites infesting *Apis cerana* had similar reproductive initiation success, because infested individuals would be removed. Consequently, affected brood in *A. cerana*, was not able to reach maturity, supporting the idea that resistance is based on behavioral traits (Lin et al., 2018).

A *V. destructor* surviving *A. mellifera* population from Norway was analyzed in order to find traits which helped reduce the reproductive success of *Varroa*. A 10% shorter than normal post capping period was found to differentiate resistant colonies from susceptible ones (Oddie et al., 2018). Spermatozoa capacitation in inseminated mites takes 5 days. This could explain, as the phoretic phase is not vital (Ruijter, 1987), why a shortened post capping period would be problematic for *Varroa* (Häußermann et al., 2016).

CONCLUSIONS

Although behavior traits seem to offer a complete explanation of defense mechanisms for *Varroa* resistant honey bees (i.e. *Apis mellifera scutellata)*, most comparative studies link resistance to physiological traits. While it is currently unknown what the molecular basis for resistance against *V. destructor* is, studies suggest that interferences in the moulting hormone biosynthesis are a likely cause. Further research is needed to fully understand these mechanisms.

Additionally, in order for the two species, *A. mellifera* and *V. destructor,* to coevolve and create a balanced host-parasite relationship, selective pressure needs to be applied. The success of breeders in obtaining resistant *A. mellifera* populations should inspire global programs of resistance-based selection.

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