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APICULTURE

Varroasis and Defense Mechanisms of Honey Bees (*Apis mellifera*)

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ABSTRACT

Background: Honey bees are threatened by mite *Varroa destructor*, which among other factors, causes what is known today in the world as Colony Collapse Disorder. The aim of this review is to publish updated information of Varroasis in *Apis mellifera*, as well as to study some defense mechanisms of bees during their co-evolution with the parasite.

Methods: The databases of Science direct, Google-scholar, Scopus, and NCBI were reviewed under the following key words: *Varroa destructor*, biological cycle, bees, Apis, Africanized bees, apiculture, and *Apis mellifera*. Special emphasis was paid to papers published within the last five years.

Results: The features of the acarid and its biological cycle, its effects on bee colonies, and the factors that favor the presence of the parasite, were described. Moreover, mechanisms like hygienic behavior, grooming, and suppression of mite reproduction were referred to. The impact of Varroasis worldwide was updated.

Conclusions: Certain bee populations manage to live with mite varroa, because their defense mechanisms allow for infestation rates at permissible ranges. The current trend is to include these mechanisms in breeding programs.

Key words: *Varroa, Apis, Africanized bees, apiculture*

INTRODUCTION

The existence of bees is currently being threatened by multiple factors, such as pesticide use, fragmentation, and the loss of habitats, along with the presence of pathogens and parasites (Sánchez-Bayo *et al.*, 2016). Accordingly, *Varroa destructor* is probably the main enemy of bees, and it is the most significant threat to apiculture in the Americas (Gutiérrez and Bautista, 2016; Reyes, 2016; Giménez *et al.*, 2017), which is also spreadout in other parts of the world (van Der Zee *et al.*, 2015; Smart *et al.*, 2016; Steinhauer *et al.*, 2018). This parasite drastically reduces the production of bee honey and other colony-made products (Khongphinitbunjong *et al.*, 2016), which leads to mid term deterioration and collapse.

However, after the unfavorable impact undergone by *Apis mellifera* following its first encounters with the mite, the bees managed to develop certain defense mechanisms. The aim of this review is to update information of Varroasis in *Apis mellifera*, as well as of some defense mechanisms of bees during their co-evolution with the parasite.

DEVELOPMENT

Varroasis in bees

Varroasis is a parasitosis caused by mite *V. destructor* (Anderson and Trueman, 2000). Originally, it only affected its natural host *Apis cerana* (Nazzi and Le Conte, 2016), but it spread out into *A. mellifera* colonies after introduction in Asia. It is considered the most significant etiological agent of beehives, because of the damages it causes and its widespread distribution (Roberts *et al.*, 2015), which also the productive indicators (Khongphinitbunjong *et al.*, 2016). The significance of losses of wild and domesticated species caused by the acarid (Smith *et al.*, 2014; Kielmanowicz *et al.*, 2015) in Europe and North America called the attention of quite a few researchers (Steinhauer *et al.*, 2018).

Molecular studies demonstrated that the European bees had been parasitized by another species of mite different from the species described by Oudemans in 1904, which was named *V. destructor* (Rodriguez, 2016). This parasite is considered one of the most influencing factors causing the colony collapse disorder in beehives (Forfert *et al.*, 2015; Lightbody *et al.*, 2016). In *A. cerana*, the mite does not cause serious damage because it only breeds in the cells where drones are bred, but *A. mellifera* breeds in both drone and worker cells (Beaurepaire *et al.*, 2015). Scientists know about the existence of two haplo-groups capable of infecting honey bees, named Korean (K1) and Japanese (J1) (Locke, 2016b).

Varroa destructor damages the tegument of bees, making them more prone to bacterial, fungal, viral, and other diseases (Kuster *et al.*, 2014). It leads to failure of the the immune (Abbo *et al.*, 2017), affecting honey bees physically and physiologically (Annoscia *et al.*, 2015).

Biology of acarid varroa (Varroa destructor)

The life cycle of this mite is tightly adjusted to and highly dependent on the bee's (Mondet *et al.*, 2014). *Varroa destructor* has morphological aspects that prove their high adaptability to parasitism: a flattened spherical shape; location of limbs on the anterior half for better adhesion to the host; sperm maturation takes place in the sperm libraries of the female's reproductive apparatus; some extracts from larvae cuticles and food in the sensory organs stimulate ovoposition and mite attraction (Dietemann *et al.*, 2013).

Cepero (2016) said that there is a marked sexual dimorphism (haplodiploidy) among *V. destructor* mites; the females are diploids and the males are haploids. The size of adult female mites is 1.0-1.2 mm long, and 1.5-1.6 mm wide, which can be seen with the naked eye (red-brown sclerotic cuticle), flattened, oval, and on four legs (Rosenkranz *et al.*, 2010).

The adult male varroa mites are smaller than females (0.7 mm long by 0.7 mm wide), pale or light brown. They are generally present only in capped broods, and copulate with the females before dying inside the brooding cells (Cassian *et al.*, 2014).

In the colonies of social insects, recognition processes are based on the expression and perception of specific chemical compounds, fatty acid esters (Cappa *et al.*, 2016), and predominantly cuticular hydrocarbons (HC). HC cover the body surface of each individual and acts like intercommunication signals to all the members of the colony (Nazzi and Le Conte, 2016); therefore, if the presence of the parasite affects the HC of the host, it could be a sign to identify parasitized individuals.

The mite uses chemical signals from the host to foster reproduction within a colony and its transmission among bee colonies, using the host's HC to find the most suitable larval stage to infest (Pernal *et al.*, 2005). This highly adaptative system is the strategy that allows the mite to optimize its search to spot the best larvae to infest, and select the proper carrier during the phoretic phase (Xie *et al.*, 2016).

The mite also mimics the chemical profile of the host´s HC, with the parasite´s HC, which is qualitatively similar to the host's (Le Conte *et al.*, 2015).

The biological cycle of the mite is made of two phases: phoretic (outer), and reproductive (inner) (Fig. 1)

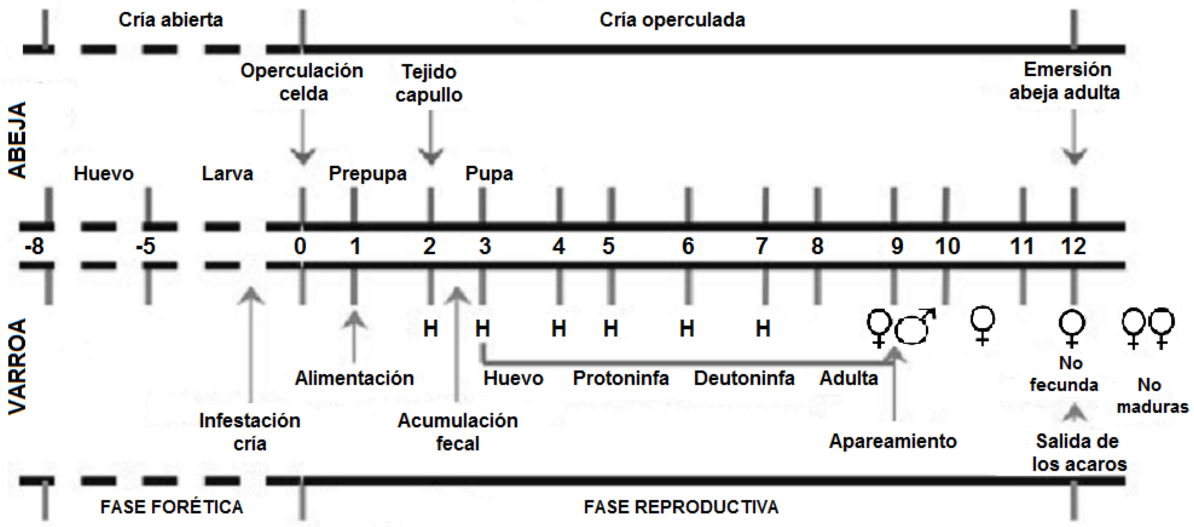

Fig. 1. Synchronization of the development cycle of varroa with the development cycle of bees. Between the two lines in the middle, is the number of days; day 0 was the occurrence of cell capping. Bee development is shown on the top. Varroa development is shown on the foot. H=egg laying (Vandame, 2001).

The phoretic stage is the period of time when a fertilized female is on top of adult bees or drones, 7-8 day incubation, until several months, mainly depending on the presence or absence of broods in the bee combs (Nazzi and Le Conte, 2016).

The reproductive stage begins when one or several fertilized females of the mite enter the cell inhabited by the workers or drones, before it is capped. The factors causing varroa to settle in the cells are believed to depend on chemical components of the hormonal nature of the larvae, which influence on the entry of mites into the cells (Salamanca *et al.*, 2012). This infestation interferes with cuticle development, thus affecting the HC profile of emerging working bees, which evolve alongside the parasite (McDonnell *et al.*, 2013).

A founding verroa may introduce an average of three breeds in the larvae or workers, and 4-5 drones, depending on the honey bee genotype (Khongphinitbunjong *et al.*, 2014). The first laying of eggs takes place between 60 and 70 h after cell capping, the remaining eggs are laid at 30 h intervals each (Calderón *et al.*, 2014). A male emerges from the first egg, the rest are females. The males reach sexual maturity before the females, and lodge at the fecal accumulation site until the first female becomes adult (Rosenkranz *et al.*, 2010).

The mite will go through several stages of development: egg, larvae, pre-pupae, and young adult, in approximately 5.8 (females) and 6.6 days (males) (Rehm and Ritter, 1989). The immature forms of the mite cannot go through the surface of the pupae's body to suck its hemolymph. Hence, they use a "feeding site" consisting in a single hole located in the ventral part of the fifth segment of the pupae's cuticle, perforated by the founding female. This encourages mites to feed by turns inside the cell (Garrido-Bailón, 2012).

The reproduction of the mite can only take place inside the cell; therefore, the males start mating as soon as the first female appears (Cepero, 2016). Hence, the duration of the stage following capping of brooding cells and the mortality of the mite's offspring in these cells are two factors that may influence on the success of reproduction (Ardestani, 2015). Mites are considered non-reproductive when they die in the cells without breeding, they fail to produce offspring, they only produce males or offspring that does not reach maturity before the bee pupae is born as an adult (Harbo and Harris, 1999).

Varroa destructor feeds from the hemolymph of developing pupae, and causes changes in their morphology and physiology (Genersch and Aubert, 2010); when the founder emerges from the cells along with the mature female offspring, they continue to feed from the adult honey bee, though Ramsey (2018) said that the mite mainly feeds from the fatty body tissue of honey bees, an organ whose function is similar to the human liver. The infestation is characterized by a reduction of the weight of emerging bees, a reduction in the bee's lifespan, deformities in their wings, legs, thorax, abdomen, and a reduction in the size of the hypo pharyngeal glands of adult bees (Froylán *et al.,* 2011).

Current impact of Varroasis on bees (Apis mellifera)

In recent years, bee populations have undergone large regional fluctuations and reduction (Dietemann *et al.,* 2013), which is a problem for productions that require insect pollination and sustainable bee keeping (van Der Zee *et al.*, 2015).

Mites may spread from bee to bee and colony to colony, through long distances, using different ways, along with migrations of colonies, routine manipulations in the apiaries, and wandering bees (Cassian *et al.,* 2014). Moreover, pests spread out due to difficulties in their eradication, inappropriate bee manipulation, and fast reproduction. Furthermore, this mite is a vector of several infectious agents.

The mortality of colonies induced by varroa is known to be caused by secondary viruses and infections transmitted by the mite (Locke *et al.,* 2014). These diseases are the probable cause of the collapse disorder. The European honeybee colonies in Europe, Asia, and North America, are undergoing massive losses, compared to bees from certain parts of the world, which had successfully survived the pathogen (Coelho *et al.*, 2015).

Hybrids from different species of bees have distinct behaviors, with certain tolerance and resistance to the mite, suggesting that some bee species are able to utilize defense mechanisms to fight infestation successfully (Harbo and Harris, 2005). The severity of the impact on bee haplotypes in certain areas is unknown; some studies correlating the genotypes indicate their dependence on the fertility of the parasite in different scenarios (Salamanca *et al.*, 2012).

Akinwande *et al.* (2014) noted that the application of chemicals to manage bee diseases and parasites has negative effects. Reports have been made on the loss of natural immunity, an increase in the susceptibility to agrochemical toxicity, and synergic effects of diseases and chemical insecticides in and out the hive. Additionally, the flying capacity of bees is reduced as a result of varroa's contact with insecticides (Blanken *et al.*, 2015).

Cruzat and Baasch (2016) said that chemical acaricides may contaminate the products in the hive, such as honey and wax, with residues that might affect consumer health. These chemicals are a burden to farmers' pockets, thus increasing the costs of production.

However, the industry needs a solution to keep their colonies producing with low infestation levels. One could be the implementation of integrated management methods to treat varroa, in order to reduce the application of chemical acaricides.

That way, the only possible solution against Varroasis is the identification and utilization of resistant populations of honey bees. More resistant bees could be achieved through better hygiene and cleaning, without losing the productive and reproductive traits of the colonies (Vaziritabar *et al.*, 2016).

Recent reports evaluate the tolerance of honey bees to the mite, and attribute their tolerance to better hygienic behavior (Coelho *et al.*, 2015). Thorough studies must be encouraged tackling the ecological interactions of the parasite, to lay out strategies of more sustainable management of this parasite, such as interrupting the mite's capacity to detect bees.

Factors influencing the rate of infestation

The mite's invasion rate to broods is a research-pending issue; however, knowing such rates is valuable information to simulate the growth of *V. destructor* populations, and therefore, it could be included in bee selection programs of resistance to mites (Vaziritabar *et al.*, 2016).

The rate of infestation can be influenced by various factors. Some of them include species´ susceptibility, geographical location, temperature, humidity, land use, pesticide burden, or availability of resources (Giacobino *et al.*, 2014; Beaurepaire *et al.*, 2015).

Additionally, the European honey bee has been described as being twice as more attractive to *V. destructor* than the Africanized bee (Coelho *et al.*, 2015). However, the latter is more resistant to certain diseases due to factors like hygienic behavior (Nganso *et al.*, 2017), which is four-fold higher than the European bees. The Africanized hybrids are more efficient mite pickers, and have lower susceptibility to invasions (Medina-Flores *et al.*, 2014), less mite attraction to the broods, the number of sterile mites in the cells of workers, and capping time in the workers' cells (Bahreini and Currie, 2015).

In turn, Vaziritabar *et al.* (2016) pointed out that the environmental conditions play a key role in the development of varroa mites. Nevertheless, this is more likely to be observed through the indirect effect of environmental factors that regulate the amounts of broods or the activity of certain behaviors in defense of the host. Some countries have been reported to have slower mite infestations, such as South Africa, where acaricides are not used (Seeley and Smith, 2015; Peck *et al.*, 2016).

Biological mechanisms, or defense strategies of the honey bee

Some *A. mellifera* populations are known to use mechanisms that allow them to live with mites for longer periods, without acaricide treatments in the hive (Strauss *et al.*, 2016). These contribute to a reduction in the prevalence of infectious diseases, and to maintain low infestation levels of ectoparasites like varroa.

The most useful mechanisms to be included in selection and breeding programs are hygienic behavior, low brood attractiveness, and suppression of mite reproducibility, though there are others which are hard to evaluate and the absence of reliable heredity (Verde *et al.*, 2013). The defensive behaviors of bees vary among species and caste of bees; also, quantification of the trait depends on the methods chosen for implementation.

Bee grooming helps them remove mites from their bodies, using their legs, jaws or shaking, biting them, rubbing their surface, and causing harm so the mites are finally gone (Pritchard, 2016).

Hygienic behavior is a highly inherited genetic trait (> 0.5), so it could be considered as part of breeding programs of *A. mellifera* in order to improve the vitality of the broods (Lin *et al.*, 2016). This kind of behavior is assumed by the skilled workers to detect mites, thanks to higher olfactory capacity (Plettner *et al.*, 2017), which helps them differentiate normal from abnormal broods with less intense stimuli (Xonis *et al.*, 2015), emerge, and remove sick broods (dead or parasitized) in the cells of hives, from the brooding chamber to the external part of the colony (Rothenbuhler, 1964a; Vaziritabar *et al.*, 2016).

The speed at which a colony removes dead broods is correlated to its capacity to eliminate the sick specimen and the parasite (Akinwande *et al.*, 2014). However, the removal of the infested broods does not necessarily include the death of the mite, most mites escape during the process of removal (Vaziritabar *et al.*, 2016); hence, it is just an interruption of the reproductive cycle of the mite, that ultimately, might delay the growth of the mite population in a colony.

Arathi *et al.* (2000) noted that hygienic behavior is predominantly done by middle-aged workers, which do not forage, and that 18% of the bees in the colony are actually involved in this at a given time. Hygienic behavior, according to Rothenbuhler (1964b), is controlled by two individual recessive genes; one is responsible for removing the sick broods from the cells (gene u), and the other is responsible for removing the sick brood out of the brooding nest (gene r); homozygosis allows bees to be more hygienic.

Later, Arechavaleta-Velasco *et al.* (2011) using RPAD molecular markers, showed seven loci with quantitative traits (QYL), that influence on the expression of this behavioral trait in the hive.

However, some questions in relation to hygiene are still unanswered, including the influence of extrinsic factors, such as the condition of the colony, available food, and type of hive. Moreover, the expression of hygienic behavior may be influenced by factors like colony space demands, structure, composition of worker´s age, environmental factors, and bee keeping management techniques (Büchler *et al.*, 2013), as well as the proportion and age of the bees that perform hygiene activities, the input of nectar and pollen collected by workers, and the strength of the colony. Nevertheless, some authors point out that the key factor is their genetic component (Medina-Flores *et al.*, 2014).

Some research reveals that bees depend on self-defense or natural resistance to fight diseases and pests (Akinwande *et al.*, 2014); hence, the evaluation of hygienic behavior is the key to tolerance and general resistance of bees.

Hygienic behavior is evaluated through several methods, such as removal of *V. destructor*-artificially infested broods (Vaziritabar *et al.*, 2016), freezing a section of the hive with the capped broods, using liquid nitrogen (Spivak and Downey, 1998), sacrificing the broods with a needle or pin (Newton and

Ostasiewski, 1986). This last method is recommended in Europe as a standard to follow in selection programs, since it shows a significant correlation to the removal of varroa-infested broods. It can be standardized and it is easy to handle by bee keepers (Büchler *et al.*, 2013). Furthermore, puncturing is more efficient; after stinging the larvae, the body fluids are shed, leading to emergence of broods and removal.

Suppression of the mite's reproductive success in the brooding cells of *A. mellifera* is another critical mechanism to achieve adaptative resistance (Mondragón *et al.*, 2006). It translates into low fertility and reproductive success of mites, and it explains the resistance shown by honey bees toward mites of various populations. Hence, there is *A. m. scutellata* in South Africa (Strauss *et al.*, 2016), Africanized bees in Brazil (Giacobino *et al.*, 2014); European bee populations in certain parts of the world, like the Island of Fernando de Noronha, northeast of Brazil; *Apis m. ligustica*, the Russian bee in the Primorsky area (Rinderer *et al.*, 2001); and in Gotland-Sweden (Lattorff *et al.*, 2015; Locke, 2016a), populations of Norwegian honey bee populations (Oddie *et al.*, 2017).

CONCLUSIONS

The solution to sustainable control and treatment of varroa is linked to the knowledge of the mite's biology, and the innate mechanisms of defense that allow bees to tolerate the presence of the parasite.

The bee populations resistant to the mite offer valuable information, and bring hope to a sustainable solution through bee resistance to the parasite. These defense mechanisms should be included in selection and genetic breeding programs, in order to create pest tolerant or resistant bees.

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CONFLICTS OF INTEREST

None