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Etiology, symptoms and prevention of chalkbrood disease: a literature review

Etiologia, sintomas e prevenção da doença cria giz: uma revisão bibliográfica

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ABSTRACT

The fungus *Ascosphaera apis*, responsible for causing the chalkbrood disease of honey bees, is widely present in temperate regions of the northern hemisphere, but has also spread to other regions of the world such as Brazil. Although it is not usually lethal for the colony, it can reduce its population, hampering its development. This study is a review on the disease that presents a broad overview of its development, identification methods as well as ways to control it. Research shows that chalkbrood is associated with several factors and is most frequently found in colonies of *Apis* bees during the spring, when there is excess humidity and sudden temperature changes in the hive. Other factors such as viral or bacterial infection, the presence of the ectoparasite *Varroa destructor*, pesticide poisoning and poor nutrition of nurse bees can also affect its incidence and severity. Field diagnosis is made based on the presence of hardened mummified brood in the pupal stage, of white or black color, in the cells and entrance. Affected cells show dead pupae covered with white mycelia, resembling cotton, or hardened, dry and brittle, resembling chalk pieces, which originated the name. To date, there are no efficient methods to reduce the damage caused by chalkbrood. Genetic selection of bees with higher hygienic behavior and disease resistance is recommended.

Key words: *Apis mellifera*, *Ascosphaera apis*, bee pathology, chalkbrood

RESUMO

O fungo *Ascosphaera apis*, responsável por causar a doença apícola cria giz, ocorre amplamente nas regiões temperadas do hemisfério norte, estendendo-se a outras regiões do mundo como, no caso, do Brasil. Normalmente não chega a exterminar a colônia, pode reduzir a sua população, prejudicando o seu desenvolvimento. O objetivo deste trabalho foi realizar uma revisão sobre essa enfermidade, apresentando um amplo panorama sobre o seu desenvolvimento, métodos de identificação, bem como as formas de combatê-la. Estudos mostram que essa doença está associada a diversos fatores, sendo mais frequente em colônias de abelhas *Apis* na primavera quando ocorre excesso de umidade e trocas bruscas de temperatura na colmeia. Outros fatores como infecções por vírus, bactérias e a presença do ectoparasita *Varroa destructor*, envenenamento por pesticida e má alimentação das abelhas nutrizes também podem induzir a sua incidência e severidade. O diagnóstico de campo é identificado pela a presença de crias mumificadas na fase de pupa endurecidas de cor branca ou negras nos favos e no alvado. As células de crias operculadas nos favos apresentam pupas mortas cobertas por micélio branco

semelhantes a algodão ou endurecidas, secas e quebradiças, semelhantes a pedaços de giz, o que deu origem ao seu nome. Até o momento, não existe uma forma eficaz para reduzir os prejuízos da cria giz e recomenda-se a seleção genética de colônias que apresentam maior comportamento higiênico e maior resistência a doenças.

Palavras-chave: *Apis mellifera*, *Ascosphaera apis,* ascosferiose, patologia apícola

INTRODUCTION

Chalkbrood is a disease affecting the brood of *Apis mellifera* L. bees. The mycosis occurs virtually all across the world, though mainly present in the temperate regions of the northern hemisphere (BAILEY & BALL, 1991). It is caused by the fungus *Ascosphaera apis*, first identified in *Apis* bees in 1913 by Maassen, who named it *Pericystis apis* (Maassen, 1913) and then reclassified it as *Ascosphaera apis* in 1955 (SPILTOIR & OLIVE, 1955). This fungus is characterized for being an opportunistic pathogen and causing mortality in broods during the pupal stage (KLINGER et al., 2013), thereby weakening the colony and reducing its capacity (EVISON, 2015).

The disease affects all castes of honeybee broods (DE JONG, 1976), which are infected after ingesting food contaminated with spores of the fungus *Ascosphaera apis*. The development of this fungus in the colony depends on a physiologically imbalanced medium or a stressful situation involving the brood during its development (ARONSTEIN & MURRAY, 2010). Chalkbrood is established in colonies infected by other diseases or by stress situations, aggravating bee mortality (POTTS et al., 2016), and hence the importance of a higher knowledgement on its symptoms, forms of diagnosis and prevention and control techniques. The present study is a literature update from a selection of the publications considered most representative of the topic in order to provide a broad overview of the etiological and morphological traits of *Ascosphaera apis*, factors causing its spread, symptoms, diagnosis and ways to prevent it.

ETIOLOGICAL AND MORPHOLOGICAL TRAITS

Genera of the fungus *Ascosphaera* are among the *Apis mellifera* pathogens. Twenty-eight species have been recorded around the world, most of which are saprophytes found in the pollen, honey and feces of larvae and in the wax within the colony (WYNNS et al., 2012). However, only one *Ascosphera apis* species is associated with chalkbrood disease.

The application of new DNA-based fungal taxonomy methods has recently provided a new update to the mycological systematics, with the current taxonomic line of the chalkbrood fungus being reclassified as *Ascomycota*, *Pezizomycotina*, *Eurotiomycetes*, *Eurotiomycetidae*, *Onygenales*, *Ascosphaeraceae* and *Ascosphaera apis* (LUMBSCH & HUHNDORF, 2007).

Ascosphaera apis is a heterothallic fungus, that is, a dioecious species (separate sexes) with two distinct mycelial forms that can reproduce sexually after interacting with another individual of a different sex (ANDERSON & GIBSON, 1998). Sexual reproduction results in the formation of fruits called fruiting bodies, which are usually 47-40 µm in diameter, depending on factors such as

temperature, moisture and culture medium used (ARONSTEIN & MURRAY, 2010; JENSEN et al., 2013).

According to Bissett (1988), those fruiting bodies are grey or brown, globose and contain sac-like spore balls 11-17 µm in diameter. Spore balls are produced in large amounts and, when mature, their color changes from white to brown and, finally, black.

The spore balls are coated by a thick transparent layer, and within them are the hyaline ascospores (1.2 × 2.5 µm), which have an ellipsoidal shape, containing several ribosomes and mitochondria. These provide the ascospores with great resistance to extreme temperatures, UV radiation and various types of disinfection products, allowing their survival for many years in the environment (ARONSTEIN & MURRAY, 2010). This disease affects worker bee larvae, drones and, at a lower frequency, the queen. Even though ascospores can affect all brood castes, susceptibility to the infection depends on the age of the larva.

Li et al. (2018) analyzed the morphology and the ultrastructure of spores of the *A. apis* CQ1 strain from southwestern China and found that mature spores are oval and long, with an average size of 2 × 1.2 µm, and firmly packed within spherical spheres. Ultrastructural analysis revealed that mature spores have two nuclei of distinct sizes. A large nucleus, with double nuclear membranes, was found within the spore, whereas the small nuclei, with only one-fifth of the volume of the large nuclei, was located near the end of the spore. The spore wall consists of an outer electron-dense surface layer, an electron-lucent layer and an inner plasma membrane. Chitin was also found to be the main component of the spore wall.

As stated by De Jong (1976), the best time for the spores to germinate are the first four days of age of the larvae. Jensen et al. (2009) showed that, in five-day-old larvae, time is a limiting factor for the germination and penetration of the spores into the larva's intestine. However, Gilliam, Taber, & Bray Rose (1978) also suggested previously that three- and four-day-old larvae are also susceptible to the fungus.

The larvae are infected after ingesting food contaminated with spores of *A. apis* that germinate in the lumen of their intestine, initiating the development and growth of mycelium in the terminal portion of the brood's intestine (HEATH & GAZE, 1987; LOPES et al., 2015). In this stage, the intestine of the larvae is closed in the caudal portion until the cell is capped (JAY, 1963). This causes the food ingested by the brood to accumulate in the mid-intestine, where the fungus spores may also be found occasionally. Under normal larval development conditions, the food (so the fungus) does not accumulate in the intestine, since, soon after capping the cell, it will be fully developed (midgut and hindgut are connected) and finally have its end opening, allowing the elimination of feces along with the spores.

However, any abnormal or stressful situation for the brood may delay larval development (the intestine will still be closed in the caudal portion) and the evacuation of feces with the spores also delayed (JAY, 1963). This fact, coupled with the presence of CO_2 accumulated in the intestine of the larva and the enzymes produced by the fungus, causes lesions on the intestinal membrane, thus providing adequate conditions for it to germinate and initiate the formation of the germ tube and the hyphae (THEANTANA & CHANTAWANNAKUL, 2008).

The fungus produces mycelia formed by irregularly septate white hyphae with an average diameter of 4.5 µm. These hyphae penetrate the peritrophic membrane of the mid-intestine of the larva, killing it, spreading throughout the body and soon initiating the production of fruiting bodies (spore cysts), giving rise to a new generation of spores (JENSEN et al., 2013; SARWAR, 2016).

FACTORS THAT FAVOR THE SPREAD OF THE FUNGUS

Experiments show the great sensitivity of bee broods when exposed to a drop in temperature to values below normal within the hive (32-35°C) for a prolonged period (> 2 h), after the capping has been sealed (BAILEY & BALL, 1991). It only takes the colony brooding area to cool for adverse conditions or stresses to trigger the disease in the larvae, since the ideal temperature for the development of fruit bodies is approximately 30 °C (ARONSTEIN & MURRAY, 2010).

In cold regions, this brood cooling may occur naturally, when the fall or winter seasons begin and there is a sudden temperature decrease. Another possibility is in early spring, when the queen lays many eggs and there are not yet enough worker bees to produce heat and keep the brood warm (MORAWETZ et al., 2019).

According to Fries (2010), Goulson et al. (2015) and Dosselli et al. (2016), additional factors such as exposure of the foraging bees to pesticides and to the microsporidium *Nosema ceranae* may predispose the colony to diseases. These causes may also be associated with the same effects of cooling and increase disease severity as well as infection by viruses, bacteria and by the ectoparasite *V. destructor*. Puerta et al. (1994) mentioned that, in tropical regions, chalkbrood can be triggered by the low temperatures at night, with the drone larvae being subjected to greater risks of cooling, as they are located in the periphery of brood combs.

Inadequate handling practices by beekeepers, such as the transfer of combs between colonies, especially if a colony is weakened, may also be related to transmission of the fungus *Ascophaera apis* between colonies (VOJVODIC et al., 2011; SIMONE-FINSTROM et al., 2018).

Padilla et al. (2014) mentioned that other factors can also contribute for the spread of the disease, such as opening the hives at low-temperature times, inducing the queen to lay eggs or dividing the colonies with artificial feed when the bee population is still low and its ability to maintain the thermal homeostasis of the colony—which is essential for its survival—is low. Factors such as sudden changes in temperature, humidity and poor ventilation in the colony predispose to the growth of the fungus, especially if there are few adult bees (NATSOPOULOU et al., 2016; NAZZI & LE CONTE, 2016).

Arranging the hives too close to each other may induce drifting of contaminated foraging bees upon return, spreading the disease in the apiary. Although adult bees are not susceptible to the disease, they may transport the fungus *A. apis* upon performing trophallaxis during the foraging stage, contaminating the nursing bees, which, in turn, contaminate the larvae by feeding them (ARONSTEIN & MURRAY, 2010). Contaminated drones are another possible direct contamination source, since they do not possess glands of identification and may enter healthy hives, contaminating them.

Food sources highly frequented by bees are also a risk factor. As stated by Castagnino et al. (2006a), the import of honey and pollen with the presence of *A. apis* spores from locations where chalkbrood is frequent might have introduced and disseminated it to regions where the disease does not yet exist. The visit of foraging bees to contaminated flowers as well as collective drinkers and feeders and other sites highly accessed by bees has been mentioned as a possible cause of contamination (KOENIG et al., 1987), though possibly of less importance.

Another noteworthy fact is that the ascospores of the fungus *A. apis* are resistant to environmental factors, possibly surviving long periods in unfavorable conditions such as high salinity, extreme temperature and sunlight. For those reasons, it is often difficult to eliminate the spores once it is established in an environment (GABRIEL et al., 2018).

The fungus spores can also contaminate honey, the melted wax and stored pollen. This highlights the risks of beekeepers when using these products from non-certified sources.

SYMPTOMS IN THE BROOD

The disease symptoms in the colonies can be seen by the presence of mummies in front of the entrance on the floor or on the ground, beneath the hive (HEATH, 1982). Perforated cappings with mummified broods and empty cells can be observed in the combs (Figure 1), indicating removal of dead brood by the hygienic behavior of worker bees (SARWAR, 2016).

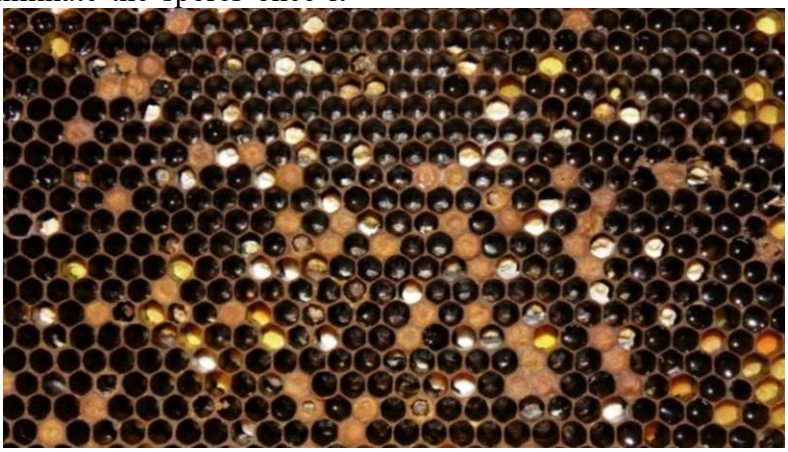

Figure 1. Pupae of mummified bees (white and black) within the brood combs.

According to Bailey & Ball (1991), infected larvae generally die within the first two days after being capped, in the pupal stage, and, soon after dying, they dilate and start to be covered by the white mycelia of the fungus. In the subsequent stage of the disease, some pupae remain white, resembling a piece of chalk (hence the name chalkbrood), while others start to turn brown or gray and, finally, black, due to the production of fruiting bodies that may vary in size or color (Figure 2). In this development stage, the mummified pupae are found enlarged, hardened and brittle, in the vertical position.

Figure 2. Magnified image showing dead pupae; a white mummy (third) still without spores fruiting and a black mummy with the presence of spores.

DIAGNOSIS OF THE FUNGUS *ASCOSPHAERA APIS*

Laboratory diagnosis is obtained by visualizing the dead pupa (mummy) under a stereoscopic microscope with 200A magnification (ANDERSON & GIBSON, 1998). Spore balls can be seen in black mummies (with spores) and in white mummies when they only have mycelia. Through this observation, the diagnosis can be made to visually identify other fungi of the genus *Aspergillus* that occur sporadically in the colonies but produce symptoms similar to those of *A. apis*, though of lesser damage to the colony. It can also be observed that the reproduction of *A. apis* is sexual and that the result of the reproduction of the fungus mycelia with the opposite sex will form spherical bodies called ascospores (spores).

Unlike other pathogenic fungi of insects that form asexual conidia, which are the infectious units of the fungus *Aspergillus*, those conidiophores are situated at the extremities of the hyphae rather than in the mycelial mass, as occurs in chalkbrood (FLORES et al., 2000). Since *A. apis* performs sexed reproduction, some authors have observed different formations of different types of strains (LEE et al., 2013). Diverse culture media can be used to cultivate the fungus, but it develops very well in media with high sugar contents such as Sabouraud agar (RAPER & FENNELL, 1965).

Initially, to prevent contamination by other types of microorganisms, the contaminated pupa is treated with a 1% hypochlorite solution and, subsequently, washed three times with distilled water. The culture medium should be in an environment with an average temperature of 30 ºC and 40% moisture (ARONSTEIN & MURRAY, 2010), forming dense and white mycelium that contains aerial, septate, hyaline hyphae 2.5-8 µm in diameter. These mycelia present superficial hyphae with very pronounced dichotomous ramifications (JENSEN et al., 2013).

For more than 15 years, new and more precise methods of identifying the presence of *A. apis* have been applied based on molecular diagnostics using the segment of the internal transcript region (ITS) of the nuclear ribosomal repeat unit (NILSSON et al., 2008) and two pairs of primers [3-F1 and 3-R1, mentioned by James & Skinner (2005); and AscosF3 and AapisR3, mentioned by Murray et al. (2005)] as sample.

Garrido-Bailon et al. (2013) developed the PCR method, which is able to detect

multiple pathogenic bacteria in bee, such as *P. larvae* and *M. plutonius* and, simultaneously, *A. apis*. Although the complete genome of the fungus was sequenced by Qin et al. (2006), little is known about the diversity of this pathogen.

SPREAD OF THE FUNGUS *ASCOSPHAERA APIS*

Chalkbrood occurs in virtually all regions of the planet, and clinical symptoms usually appear in a short time, most commonly in cold seasons with high humidity, including hot and dry climates (ARONSTEIN y MURRAY, 2010). According to Jensen et al. (2013) and Seyedmousavi et al. (2015), in several countries, chalkbrood is considered a notifiable disease that should be reported to the authorities when observed in colonies.

Epidemiological surveys carried out in Spain in 2006 and 2007 revealed a low incidence of diseases in bee broods, with chalkbrood causing the highest mortality rates (5.0%) and surpassing the American foulbrood, caused by the bacillus *Paenibacillus larvae* (3.0%). In comparing the origin of positive samples, chalkbrood was found to be more frequent in warmer areas, whose climatic characteristics thus far have not been considered favorable for the development of the disease (GARRIDO-BAILÓN et al., 2013). This observation is in line with the results of Aronstein & Murray (2010), who mentioned that *Ascosphaera apis* appears for a short period and is generally associated with cold and high humidity climates. According to Garrido-Bailón et al. (2013), a possible explanation for this high prevalence in those regions is stress in the colonies caused by *Nosema ceranae*, which occurs more frequently in the warmer region of Spain.

Since chalkbrood is an opportunistic pathology, it finds appropriate conditions to develop in weakened colonies, regardless of ideal environmental conditions. According to Cavigli et al. (2016), this observation shows how vulnerable the colonies may be to the attack of the fungus *A. apis*, since they are constantly under the action of stress inducers such as pesticides, migration, lack of food, parasites and other diseases (SIMONE-FINSTROM et al., 2016). According to Heath (1985), from the 1970s, chalkbrood was detected in countries like Japan, the Philippines and Mexico, where it was considered the disease whose frequency most increased among *Apis mellifera* L. species (WILSON et al., 1984). In South America, it was initially reported in Argentina, in 1978, by Rossi & Carranza (1980) in the province of Buenos Aires. From that period, it spread rapidly and is currently present in all areas where beekeeping is practiced (ALBO & REYNALDI, 2010), with an incidence of around 11% in the colonies (REYNALDI et al., 2003). In Brazil, four isolated cases were initially reported: one in the state of São Paulo (ROCHA et al., 1998), two in Rio Grande do Sul (SATTLER et al., 1998; CASTAGNINO et al., 2006a) and one in Minas Gerais (CASTAGNINO et al., 2006b). Castagnino et al. (2006a) described that the fungus *A. apis* was widespread in southern Brazil from 1998, the year of its first report. The authors suggested that the introduction and expansion of the disease in the state of Rio Grande do Sul was due to the migration of colonies to cultivation fields located on the border with Uruguay and Argentina, where the disease had been frequent for many years. Teixeira et al. (2018) analyzed 41

samples of bee products marketed in the state of São Paulo and identified the presence of spores of the pathogen *A. apis* in 73.17% of them, in addition to other investigated pathogens.

FORMS OF PREVENTION AND CONTROL OF THE FUNGUS *ASCOSPHAERA APIS*

According to Wilson et al. (2015), the *A. apis* fungi are resistant to biocides like sulfuric acid, iodinated substances and other products that are commonly used to disinfect hives infected by diseases. Thus, eliminating the disease focus becomes a hard task once it is already established in the apiary.

Studies led by Starks et al. (2000) showed an additional behavior of the colony in an attempt to reduce the possibility of infection and control of *A. apis*. Bees seem to recognize the attack of this disease and react to the infestation by producing a temperature increase in the brooding area. According to those authors, this temporary heating aims to reduce mycelial growth and prevent infection of healthy larvae, since *A. apis* are sensitive and do not develop at temperatures above 35 ºC. This shows the importance of keeping colonies in suitable places and providing good-quality hives for the colony to maintain its homeostasis. However, to date, there is no effective technique to eliminate the spores of *A. apis* or a pharmacological treatment to minimize its effects on the hive (PUERTA et al., 1995).

Researchers have tried new ways to control chalkbrood by using natural products. Many of these studies, carried out *in vitro*, focused on the use of essential oils of aromatic plants in the control of *A. apis* and showed effective results, with emphasis on the oils from the plants *Litsea cubeba*, *Pelargonium graveolens*, *Croton bonplandianus* and *Mentha spicata* and compounds formed by oils of *L. cubeba*, *C. zeylanicum*, *Cymbopogon flexuosus* and *L. cubeba*, *C. zeylanicum*, *P. graveolens* and *C. flexuosus* (Saleem et al., 2015; Ansari et al., 2015; Nardoni et al., 2018). Chaimanee et al. (2017) also tested the antimicrobial activity of 37 plant extracts against *A. apis in vitro*. Seven species—*Amomum krervanh*, *Allium sativum, Cinnamomum* spp., *Piper betle*, *Piper ribesioides*, *Piper sarmentosum* and *Syzygium aromaticum*—exhibited inhibitory effect against the fungus.

Another natural product non-toxic to bees that constitutes an alternative to the use of synthetic drugs is propolis from *Apis* bees, considered an important component of immunity for the hive due to its antimicrobial activity. Wilson et al. (2015) characterized the metabolic profiles of propolis from 12 different climatic regions in the USA by comparing the antimicrobial activity of samples in culture medium against the pathogens of *P. larvae* and *Ascophaera apis* bees. The authors verified their viability to control the growth of these pathogens, but with important differences in inhibition capacity depending on the region of the United States. More recently, Wilson et al. (2017) researched the botanical drug profile of propolis compounds in North America by *in vitro* testing and proved their inhibitory activity against the same pathogens.

Simone-Finstrom et al. (2018) studied the potential of gamma irradiation for the inactivation of *Ascosphaera apis*, among other pathogens, and confirmed the effectiveness of this technique against the fungus. The authors also stressed that this treatment may help reduce colony losses and the spread of

pathogens through exchanges of combs between colonies.

Another way to control the spread of the fungus without using fungicides and avoid contamination of colony products would be the genetic selection of bees with greater hygienic behavior. Gilliam et al. (1988) found that hygienic behavior is correlated with the resistance of the colonies and with the chalkbrood disease and the population of the fungus *A. apis* in the colonies. The same authors found that, in colonies that exhibited reduced hygienic behavior, there was a wide range of spores distributed in the brooding area and in the honeycombs. This finding shows that the use of selected queens, with greater hygienic behavior, should be a more usual tool for beekeepers.

According to Invernizzi et al. (2011), the advantage of more-hygienic over less-hygienic colonies is that bees detect infected larvae more quickly. They remove dead pupae from the hive before they become infectious mummies, reducing the possibility of the disease establishing in the colony. Al Toufailia et al. (2018) quantified the removal of larvae killed by freezing and infected with chalkbrood in uncapped cells in 20 colonies and concluded that there are two adaptive peaks that confer disease resistance: high hygienic behavior (the sick brood is removed quickly and, in some cases, before it becomes infectious) and poor hygienic behavior, when the sick brood remains isolated within the sealed cells.

However, Gerdts et al. (2018) found that hygienic behavior was not a significant predictor of the presence of *A. apis* infection in colonies. Lui et al. (2016) drew attention to the fact that there is not yet an easy method to select and keep resistant bees. For this reason, their study aimed to find the genes involved in the development of resistance and to identify single nucleotide polymorphisms (SNPs), which can be used as molecular markers of resistance. The results showed that the SNP C2587245T can be useful as a genetic marker for the selection of bees resistant to chalkbrood.

MANAGEMENT STRATEGIES TO REDUCE RISK OF COLONY LOSSES

In addition to negatively affecting the growth and production of colonies, pathogens and diseases cause significant economic losses for beekeepers (Panasiuk et al., 2014), and some management practices can be used to mitigate colony losses. Berry & Delaplane (2001) suggested replacing old honeycombs or those with symptoms of *A. apis* as an initial management technique to eliminate spores from the hive. The replacement with new wax in the combs has the advantage of reducing the presence of spores and inducing the queen to lay eggs, promoting an increase in the bee population. It is recommended to replace one third of the hive combs annually and to clean and disinfect equipment such as chisels, gloves and fumigator after handling sick hives. Hives with symptoms of any disease should be the last to be managed, thus reducing the possibility of healthy colonies being contaminated by the beekeeper's equipment (FLORES et al., 2000). Castagnino et al. (2006a) recommended avoiding feeding the colonies with pollen from unknown apiaries, as it can be a source of contamination and spread of pathogens.

Another recommended measure would be the immediate removal of abandoned hives from the apiary to avoid robbing and because it is a source of infectious material. Flores et al. (1996) suggested

avoiding manipulating the colonies at times of lower temperature to avoid cooling the offspring, while Castagnino et al. (2006a), Flores et al. (2000) and De Jong (1976) recommended not placing the hives directly on the ground to avoid humidity and low temperatures during the night. Another strategy, according to Castagnino et al. (2006a), would be to replace the queens of hives that had clinical symptoms of chalkbrood with queens from colonies that show a higher hygienic behavior and to avoid the migration of hives to regions where a high prevalence of disease is present (Traynor et al., 2016; Guimarães-Cestaro et al., 2017). In the summer, Evison & Jensen (2018) suggest that colonies with a small population should be fed before the winter begins to induce their increase and maintain thermal homeostasis, making them stronger and better able to recover.

FINAL REMARKS

The fungus *Ascosphaera apis* is a pathogen that attacks *Apis mellifera* bees during the pupal stage. Though not highly lethal, its impact on hives can be significant, as it reduces the colony brood. Contamination occurs when the brood eats food infected with spores of the fungus. For the fungus to affect the colony, the physiological environment must be out of balance or the brood must have faced some stressful situation during its development.

Because chalkbrood is an opportunistic disease, its presence may be an indication that the hives are infected by other diseases such as the ectoparasite *Varroa destructor*, which could greatly weaken the colonies. In this regard, the importance of knowing the symptoms of this fungus, forms of diagnosis, prevention and control techniques is reinforced.

Additional factors associated with management practices of beekeepers may be related to the transmission of *Ascophaera apis* between colonies, such as the introduction of contaminated combs from other hives. The ascospores of this fungus can also contaminate the stored honey, melted wax and pollen, which shows the importance of the beekeeper buying these products only from certified sources.

There is no consensus among the developed studies as to how to combat this fungus. For this reason, further research is warranted on more effective ways that can effectively reduce the incidence of this disease.

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