

## Fungal Infections In Honey Bees

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### Abstract

The apiculture is very important to the international agroindustry, due to the fact that bees are responsible for the highest percentage of plant pollination of human food. However currently the bee keepers are facing troubles due the loss of *Apis mellifera* colonies causing losses to the honey production and mainly to the pollination of important agricultural cultures in the world and the impacts in food security. The decline of these pollinators can be explained by the many factors like, bad nutrition, loss of the queen and many pathogens and parasites. Stonebrood is a disease caused by the fungal *Aspergillus* through the ingest of spores and through the scarf-skin, therefore, can infect larvae and adult bees. Other pathology is the Chalkbrood, an infection disease caused by the fungal *Ascosphaera apis*, that occurs in bee larvae (*A. mellifera*) is usually more prevalent in the spring, since it the growth is enhanced in cool and damp place. In recent years many researches were performed using many chemotherapeutic and chemical products looking for any alternative to the treatment of Chalkbrood, but none of these studies obtained positive results. Some strategies have been used by the beekeepers to try to control the Stonebrood and Chalkbrood like: management and sanitation practices (include a food supplementation, to improve the health of bees, keeping the hive clean and ventilated, and preventing transfer of the pests between colonies); use of natural products and ecological safety. Is important prevent the use of synthetic pesticides because can impact in bee health and make the honey inappropriate to the consume.

### Introduction

Bees play an importance role in agriculture, due to the pollination that they provide, being 52 of the 115 main global food products depend on the pollination by bees [1]. About 22.6% of all agricultural production in developing countries and 14.7% in developed countries is directly dependent on the pollination of animals to some extent [2]. The *Apis mellifera*, the European honeybee, is a highly adaptable species, being native to an area that stretched from the southern part of Scandinavia to Central Asia and Africa [3-5]. Since the 1600s, this species has expanded by almost all habitable parts of the globe, because humans have transported this important insect deliberately [6]. Pollination is performed not only by bees and in most cultures they aren't the most effective ones in this function. However, when it comes to monocultures, they remain the most important pollinator [7,8].

### Honeybee Diseases

The recent unexplained losses of bee hives, which are very important for pollination, led to a wave of public and scientific interest in relation to the pathology of the bees (*Apis mellifera*) [9,10]. Different agents can compromise the survival of the hives, such as poor nutrition, pesticides and pathogens [11]. Bees are vulnerable to a high diversity of parasites, for example, *Varroa destructor* and *Nosemaceranae* [12-14], which have attracted much attention in recent years.

Many diseases can affect bees (bacterial, fungal, viral, microsporidia), parasites (mites), predators (bears, birds, humans) or pests (beetles, moths) that can affect the productivity of honey and honeybee survival [15]. Honey increased by 58% in its production since 1961, and in 2007 we got the equivalent of 1,070,000 metric tons. The average price in 2006 for honey was \$ 1,168 a metric ton, and the total value in 2007 was estimated at \$ 1.25 billion [11].

In the kingdom of fungi, pathogenic fungi of insects can be found [16], damaging the immune system of these insects. Two genera of fungi (*Aspergillus* and *Ascosphaera*) are known because of the infection caused at litters of bees, causing the Chalkbrood and Stonebrood diseases.

### Stonebrood Disease

The *Aspergillus* spp. are cosmopolitan filamentous fungi frequently found in soil, growing as saprophytes, but occasionally they infect living hosts, including plants, insects and mammals. In humans, they can infect the eyes, lungs, pharynx, open wounds, but they are most commonly seen in immunocompromised individuals [17-21]. In addition, aflatoxins, produced by this fungus, present carcinogenic characteristics if ingested or inhaled, therefore, when this disease occurs in bees, some precautionary actions should be taken for the protection of beekeepers and consumers. In many countries, Stonebrood is a compulsorily notifiable disease, being necessary to report it to the authorities.

Stonebrood is considered a disease of low virulence for hives, however, little is known about the host-parasite system of this disease [22,23]. Some species of the genus *Aspergillus* are optional parasites and they have been reported as Stonebrood agents [24,22].

The disease was first described by Massen [25], being it found worldwide. *Aspergillus flavus* was the most reported cause, followed by *Aspergillus fumigatus*, but also *Aspergillus niger* and other species can kill honeybees [26]. The infection is based on the ingestion of spores and through the cuticle, so adults and pupae as well as larvae can be

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infected. The production of aflatoxins by various species of *Aspergillus* has been suggested as the primary cause of death of bees that presented Stonebrood [27].

The research of *Aspergillus* is mainly focused on health and human nutrition due to contamination of grain with aflatoxins. Stonebrood outbreaks are rarely observed, which may be a result of removal of infected individuals by the bee hive. Spores of *Aspergillus* spp. are found in different places and a high virulence for bee larvae was demonstrated. Even though Stonebrood is rarely reported, it is interesting to understand the factors and the mechanisms that carry out the occurrence and strength of this disease [28].

## Chalkbrood Disease

Chalkbrood is an invasive mycosis produced by *Ascosphaera apis*, which affects only the creation of honey bees, being fatal to the individual larvae, but it usually does not destroy the whole bee hive [29,30]. Whereas, it might be causing significant losses in productivity and number of bees [31,32], resulting in reduced production of honey 5-37% [33-35]. It is a chronic disease that is established in bees after they consume the spores of *A.apis* (Ascomycota). At low temperatures and high humidity in the hive, the risk of Chalkbrood outbreaks tends to increase [36].

This disease is found in bee colonies worldwide, and there is an evidence that the incidence of Chalkbrood has increased in recent years [37,38]. According to Aizen and collaborators [39], human activities related to the increased demand for food could be responsible in part for this tendency.

Both Chalkbrood and Stonebrood were documented for over a century, but much remains to be discovered in relation to their impact on bee health. Several studies related to both diseases reflect their frequency, with a difference of magnitude in favor of Chalkbrood. A recent study of database and monitoring for six years indicated that hives that present a high number of *Varroa* mites or infection by *Nosemaceranae* in the spring increase the chances of Chalkbrood outbreaks [40].

The Chalkbrood disease was recognized in the early twentieth century [41], and its diagnosis is based on the view of the brood of bees, which have a mummified form, commonly known as "Chalkbrood mummies". This disease can reduce the productivity of the colony due to the decrease in the number of bees, and in some cases, it may lead to the loss of the whole hive. The genus *Ascosphaera* spp. comprises some saprophytic species that develop in nesting materials such as stored food, fecal matter and debris from the nest, and other species act in an opportunistic way regarding bees [42].

*Apis Ascosphaera* infects bee larvae by hyphal penetration of the intestinal wall and the mycelium grows within the body cavity. After a few days, fruiting bodies with new ascospores are formed on aerial hyphae, on the outside of the dead larvae [43]. There are several predisposing causes mentioned in the literature, one of them refers to the stress caused by cooling, Flores and collaborators [36] observed that inoculated larvae were cooled to a temperature of 18-20°C in the hours before and after the alveolus cover. After the thermal stress, 100% of the pupae got sick when the temperature returned to normal (25°C), while those ones that did not experienced the cooling did not develop the disease.

Another exposed factor is the overuse of antibiotics, especially oxytetracycline, used to control and prevent bacterial diseases in

beekeeping, as the use of antibiotics in bee can change the equilibrium of intestinal microflora, favoring the growth of fungi such as *A. apis* [44]. However, Flores and collaborators [45] developed a method to study the effects of this antibiotic in different situations and concluded that oxytetracycline does not increase the risk of Chalkbrood in bee workers in short or medium term.

The hygienic behavior of bees is defined by the capacity of detection and removal of the diseased hive brood [46], this behavior is one of the factors responsible for resistance to Chalkbrood [47]. Bees that present these characteristics are insensitive to olfactory stimuli associated with the sick brood, being able to detect and remove them before becoming an infectious stage in the hive [48].

The incidence of Chalkbrood is more prevalent during the spring, once the development of fungi is favorable in cold and wet hives [36,49-52]. The severity of the disease is influenced by environmental conditions and also by the interaction between biotic factors, such as differences in fungal strains and the genetic background of bees. In recent decades, some research has been carried out for improving the lineage of bees, with regard to resistance to infectious diseases [53-58]. While the adult bees are not susceptible to *A.apis*, the transmission of the disease may occur inside the hive between the bees, by sharing food [26].

The spores of this pathogen can accumulate in the hive and also in the hive products, as the foundation wax, stored pollen and honey, remaining viable for about 15 years, being a source of long-term infection [59-64]. These spores ingested by the larvae of bees germinate in the lumen of the intestine. Then, they are probably activated by the action of CO<sub>2</sub> [65,66]. Thus, the fungal mycelium develops inside the body cavity, getting out through the rear end of the caterpillar [43,67,68].

The death of the larva is the result of mechanical and enzymatic injury, which affects circulation, in addition to the action of toxic effects [69]. In a more advanced stage of the disease, the larva is covered with a thick layer of mycelium, from the rear end to the front end. After a while, some brown or black spots appear due to production of ascomata.

## Treatment and Healing of Honeybees

In order to keep the healthy and natural characteristics of bee products, the development of alternative methods for controlling Chalkbrood and Stonebrood is needed, avoiding the use of fungicides that might contaminate the final product. It is important that beekeepers avoid transferring wax honeycombs from the beehives that present infections and spores, as the occurrence of the disease is proportional to the amount of circulating spores [70].

For a control of Chalkbrood, many chemotherapy agents have been tested [71-74]. A list of chemicals that seemed to be promising for the disease control was tested; however, they did not present the necessary effectiveness [75]. Currently several strategies have been implemented to control this disease, as the use of bees resistant to Chalkbrood, improved management and sanitation practices, and the use of ecologically safe natural products. The use of pesticides and antibiotics may deteriorate the hive and compromise the health of bees. Then, it is advisable to reduce the use of these substances [76-78].

The sterilization of honey by heat is a decontamination strategy which presented good results, but with limitations. The heating above 90°C results in caramelization and also in the growth of a harmful chemical named Hydroxymethyl furfural, besides reducing beneficial

enzymes found in honey. For this reason, a lot of studies are focused on obtaining alternative methods, such as microwave radiation, infrared heating, ultrasound and ultra-filtration in order to preserve the quality of the honey [79].

The use of natural compounds for the disease control would also be an alternative, such as products derived from plants with antimicrobial activity [74,75,80]. Some essential oils such as citral, citronella, and geraniol were tested in vitro having the best inhibitory effect of the fungus [74]. All these findings need a field study to evaluate the activity of these products in hives.

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