FACTORS AFFECTING HYGIENIC BEHAVIOUR IN *APIS MELLIFERA* HONEYBEE COLONIES: EFFECT OF *VARROA JACOBSONI*

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Abstract

The work was done at Coronel Vidal city, province of Buenos Aires on 6 honeybee (Apis mellifera) colonies. In order to determine removal rate all colonies were examined using brood perforation method. One week later, 300 Varroa jacobsoni *females were introduced in each colony and removal rates were calculated again. Results showed that parasites presented a significative effect on honeybee hygienic behavior (x^2, $p<0.05$). All colonies increased their capability to detect and remove dead brood, reaching a level of 90.7% in one colony. This genetically determined behavior would be increased upon adverse situations such as high mite infestation levels. This response would be very useful to reduce damages to the colonies.*

Key words: Varroa jacobsoni/Apis mellifera*/higienic behavior/Argentina*

Introduction

At present, the presence of the mite *Varroa jacobsoni* in honeybee colonies represents the most serious pathology for beekeeping industry[5]. This mite produced big losses in the number of colonies in Argentina – 100,000[8], Spain – 300,000[10], Poland – 2,000,000[11].

The parasites attack adult honeybees and brood where it reproduce. Mites feed on bee haemolymph, causing malformations on wings and legs[6, 17], a decrease on bee longevity (6) and transmission of pathogenic agents[1]. The phoretic phase is variable, ranging from 1 to 14 days[25]. After that, mite females penetrate honeybee worker or drone brood cells to reproduce.

Honeybee hygienic behaviour consist in detection and removal of dead brood and represent a resistance mechanism of bees against different diseases[2; 4; 3]. This behaviour is determined by two separate recesive gene[23] and the expression depends on climatic factors and colony strengh[21; 18; 19]. Hygienic capability is considered a way to evaluate the resistance to American foulbrood and chalkbrood[9]. In recent years, a decrease of mite population on hygienic colonies was observed[22; 19; 3; 14]. The aim of this work is to analyze the effect of mite infestation on *Apis mellifera* hygienic ability.

Material and Methods

The work was done in a commercial apiary located at Coronel Vidal, Mar Chiquita District, Province of Buenos Aires, Argentina, during October and November months. Six Langstroth colonies of an hybrid of *Apis mellifera mellifera* and *Apis mellifera ligustica* were selected. These colonies presented sister queens and similar number of adult bees, brood and reserves. All colonies were selected based on their proved hygienic ability[15]. Parasitic prevalence was determined in each colony before the experience beginning, using the 3 frames technique. 200 adult bees were collected from 3 brood frames and put into flasks with water ethilic alcohol (1: 1). Numbers of mites and bees were counted and expressed as proportion[16].

Hygienic capability was determined using brood perforation method. In every colony, a 10 cm x 5 cm rectangle was marked on a frame and each brood cell was perforated with a needle. After 48 hours, the frames were taken out and the number of brood removed was registered. Removal rate was calculed as the number of removed cells divided by the total number of brood cells selected.

Seven days later, 300 Varroa mites were introduced in each colony and hygienic capability was again evaluated to establish *Varroa jacobsoni* effect upon this behaviour. Kruskal-Wallis analysis between removal rate before and after artificial infestation was performed ($p<0.05$)[26].

Results

At the beginning, all colonies presented mite infestation rates very low, with a minimum value in colony 2 (1.06%) and maximum in colony 5 (2.48%; Table I; X = 1.77 ± 0.55). Under these conditions, hygienic capability was elevated. Removal rates showed little variation between colonies and higher than 75%. Colony number 3 presented highest removal rate (82.5%) and colony number 4 the lowest (75.2%; Figure 1). Correlation between parasitic prevalence and hygienic behaviour was not observed.

Table I

Number of honeybees and mites collected to determine parasitic prevalence in *Apis mellifera* colonies before artificial infestation

Colony	Number of honeybees	Number of mites	Parasitic prevalence (%)
1	215	5	2.32
2	187	2	1.06
3	236	4	1.69
4	228	3	1.31
5	241	6	2.48
6	225	4	1.77

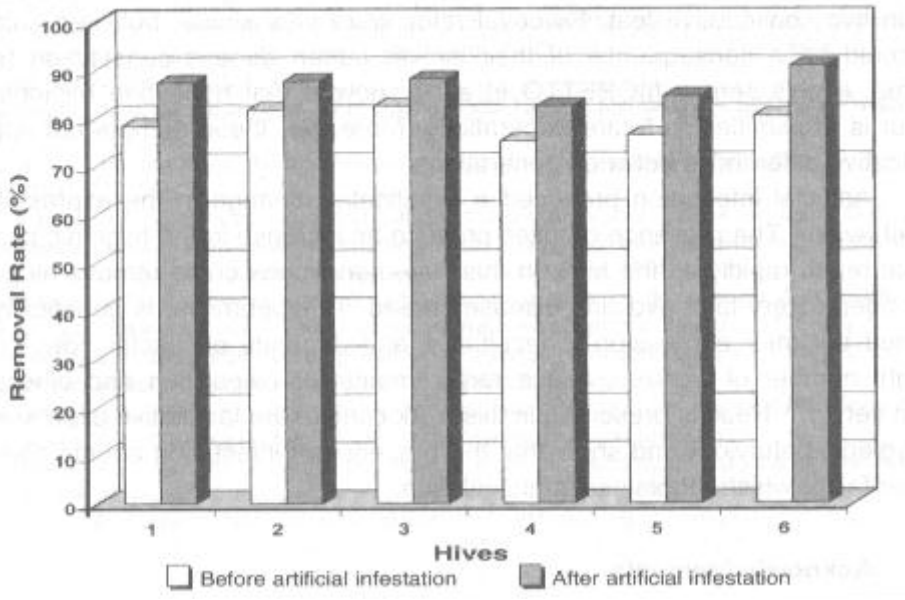

*Figure 1 – Hygienic behaviour of six honeybee (*Apis mellifera*) colonies before and after artificial* Varroa jacobsoni *infestation.*

After artificial infestation all colonies exhibited a marked increase in their hygienic capability. Honeybees removed brood cells much more successfully, with removal rates significative higher to those registered before infestation (Kruskal-Wallis, X^2 = 348; d.f. = 1; $p<0.05$, Figure 1. This increase was variable ranging from 5.6% in colony number 3 to 10.4% in colony number 6. In this latter colony, honeybees removed 90.7% of brood cells in 48 hours.

Discussion

Results presented in this work, show low infestation levels at the beginning of the experience. The presence of mites always cause damages to the colonies. However, considerable negative effects appear with parasitic prevalences higher than 8%[13]. Higher removal rates did not present correlation with lower parasitic prevalences. However, it could be thought that both factors are strongly related. Several authors showed that honeybee hygienic behaviour had a negative effect on mite infestation degree[22; 2; 19; 3]. Re-

cently, MARCANGELI[14] showed that colonies with higher expression of this behaviour presented lower parasite population levels. If a brood cell is desoperculated, inmature mites die[12] and adult mite females would search other brood cell. In consequence, this fact produce a slower mite population growth.

Initial removal rates are agree with previous results[15]. According to GILLIAM et al.[9] a colony is considered as hygienic when removal rates are higher that 70% in two consecutive test. Removal rates were very similar between colonies and could be a consequence of their similar queen genetic constitution (sister queens). In this sense, MORETTO et al.[20] showed that honeybee hygienic behaviour is transmitted to future generations. Moreover, these authors did not find significative differences between generations.

Artificial infestation produced a significative change in the expression of this behaviour. The presence of mites produce an increase in the hygienic capability, that reach rapidly all the hive. In this way, honeybees could remove all pathogenic agents very fast, avoiding disease spread. This behaviour is genetically determined but their expression is facultative and depends on nectar flow, colony strengh, number of workers, space requirements for oviposition and others unknown yet[19; 24]. Results presented in this work confirm the facultative expression of the hygienic behaviour and show that the presence of infestation agents could be another factor which regulates it manifestation.

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