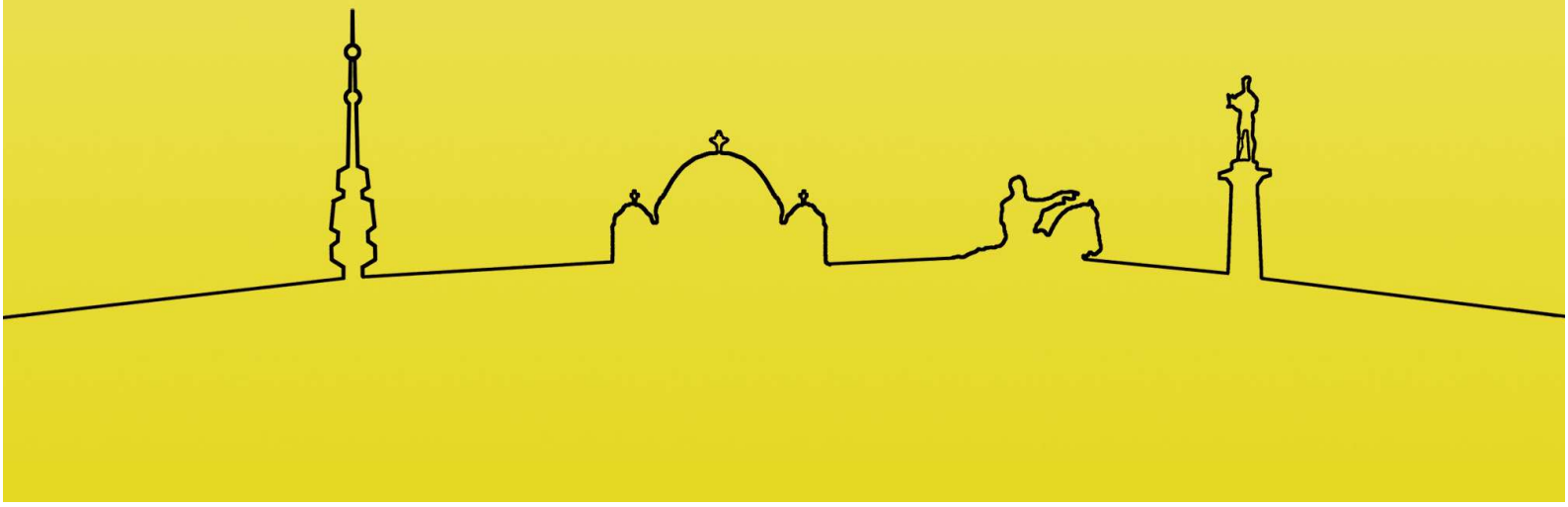
EurBee 9

9th European Congress of Apidology

20-22 September 2022

Belgrade, Serbia

Abstract Book



INTERACTIONS BETWEEN PREVALENT PATHOGENS OF HONEYBEES (*APIS MELLIFERA*)

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The aim of the BEEHEAL project was to determine the phenology and interactions of the microsporidia *Nosema ceranae* and 5 highly prevalent and pathogenic honeybee viruses (DWV, BQCV, ABPV, IAPV and CBPV) in four Mediterranean countries: France, Israel, Portugal and Spain. The prevalence and phenology of these pathogens was monitored in four continental apiaries (one in each country) and three insular apiaries located on Ouessant Island (France) and on São Miguel and Santa Maria Islands (Portugal). Colonies were sampled during a 2-year study period, and honeybees were collected and analysed once every two months. Microsporidia detection was performed by conventional triplex-PCR whereas viral loads were determined by RT-qPCR of the positive samples. Israel had the highest percentage of honeybees infected by *N. ceranae*, followed by Spain, continental Portugal, and Ouessant, whereas the levels were the lowest in France mainland and it was not detected on the Portuguese Islands. The countries with the higher prevalence of viruses were France and Israel whereas the apiaries in Portugal and Spain had a lower percentage of colonies and individual honeybees infected by viruses. Moreover, CBPV was not detected in the latter countries. The viral load of colonies and honeybees coinfected with two or more viruses was positively correlated. Data analysed from all apiaries together showed a negative correlation between the percentage of infected *N. ceranae* honeybees and the viral load. However, there was no correlation at the apiary level between *N. ceranae* and the viral load when analysed separately. Cross-tabulations showed a correlation in coinfection between *N. ceranae* with any of the three viruses: BQCV, IAPV and CBPV. In fact, honeybees coinfected by the microsporidia and one of these viruses had a higher viral load than honeybees infected only with the virus. This study showed that pathogen interactions

differs greatly among countries and serves as a starting point for the development of epidemiological studies to unravel pathogen coinfections.

Keywords: viral load, microsporidia, coinfection

FORMULATIONS OF OXALIC ACID AGAINST *VARROA DESTRUCTOR* (DOSAGE, EFFICACY, DECOMPOSITION, RESIDUES)

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The control of varroa (*Varroa destructor*) is one of the most crucial problems that beekeepers have to deal with. Usually, they apply on their bee colonies treatments including chemicals or natural substances such as organic acids. One of the most commonly used organic acids is the oxalic, which is applied through various formulations. In the present study, we evaluated the efficacy of the oxalic acid – glycerin strips method through time and identified the optimum concentration of oxalic acid in brood presence. According to the results, the optimum concentration of oxalic acid was 21% in water-glycerol solution (56.5: 43.5, v/v), applied to absorbent strips (60g oxalic acid, 100 mL H_2O, 130 mL glycerin, for 13 strips). The efficacy of this concentration was found more than 90% over a period of four years applications. In addition, the glycerin strip method was compared to others (using synthetic and organic acaricides) commonly used by beekeepers, such as vaporization and fumigation of oxalic acid. Although the vaporization showed good results against varroa, it can sometimes have negative effects on the bees, such as the loss of queens, while the chemical control led to reduced efficacy during the four years (lower than 80%). Moreover, the possible residuality of oxalic acid in honey was studied using high performance liquid chromatography (HPLC-DAD). The concentration of both oxalic acid and formic acid in honey samples was not affected by the presence of strips in the hives. Finally, it was observed that the decomposition of oxalic acid during the preparation of the strips is intense when the solution is heated to a high temperature. During the heating, apart from the decomposition of oxalic acid, there was also a simultaneous formation of formic acid and an unknown compound, which could not be identified by the present method of analysis.

Keywords: Oxalic acid, glycerin strips, decomposition, residues