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Gamma radiation: a sanitating treatment of AFB-contaminated beekeeping equipment

Alessandra Baggio, Albino Gallina, Nicoletta Dainese, Chiara Manzinello, *Giorgia Serra, *Roberto Colombo, *Emanuele Carpana, *Anna Gloria Sabatini, **Klaus Wallner, ***Roberto Piro, Franco Mutinelli

National Reference Laboratory for Beekeeping Istituto Zooprofilattico Sperimentale delle Venezie Viale dell'Università, 10; 35020 Legnaro (PD), Italy; Phone: +39 049 8084259; Fax: +39 049 8084258; e-mail: fmutinelli@izsvenezie.it

*Istituto Nazionale di Apicoltura, via di Saliceto, 80, 40128 Bologna, Italy, e-mail: agsabatini@inapicoltura.org

**Universität Hohenheim, Landesanstalt für Bienenkunde, 70593 Stuttgart, Germany, e-mail: bieneva@uni-hohenheim.de

***Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via A. Bianchi, 9, 25124 Brescia, Italy, e-mail: rpiro@bs.izs.it

INTRODUCTION

American foulbrood (AFB) is a serious disease of honeybee brood caused by spore-forming bacteria *Paenibacillus larvae* subsp. *larvae*. Although this disease is well known, AFB is still a relevant cause of beehive and economic losses to beekeepers. In fact, spores of *P. larvae* are very resistant and remain viable almost indefinitely in spore-contaminated hive equipment being a potential source of infection. Furthermore, AFB is characterized by an easy dissemination of spores within-colony and between colonies. Spores dissemination may occur naturally via swarming and robbing, but also artificially through certain apicultural practises such as use of interchangeable combs and other hive parts, movement and sale of colonies. An efficient control of AFB can be obtained only when the detection of AFB-contaminated hives is rapid and their elimination is performed immediately. About the last crucial step, burning infected colonies is an effective way of killing spores, interrupting the disease cycle. However, since this results in hives destruction, it is not well accepted by beekeepers.

A very interesting method to decontaminate AFB infected equipment and to control AFB, is the use of gamma radiation(s). The first experimental studies were carried out in the '70 which demonstrated the lethal effect of radiation treatments on AFB-spores without destructive action on equipment. However, if the biocidal action of gamma radiation(s) due to disruption of DNA is known, few information are available on the effects of gamma radiation on wax and honey. The aim of this work is to verify the sterilizing effectiveness of gamma radiation on AFB-contaminated apicultural equipment applied at different levels and, the possible effects on physico-chemical characteristics of some beehive products as beeswax and honey.

MATERIALS AND METHODS

⁶⁰Co was used as source of gamma radiation. The samples were processed in duplicate and irradiated at three different levels: 10, 15 and 25 kGray. Gamma radiation treated equipment was analysed by four different laboratories according to matrix and parameters considered.

Hive equipment. Three Dadant-blatt hives with AFB-contaminated combs were used. The infectivity of spores contained in scales was tested before and after the radiation treatment by culture.

Honey samples. Three honey samples of different botanical origin were collected: *Robinia*, Honeydew and Multifloral. 28 samples of each honey (250 g/each) for physico-chemical analysis and 14 AFB-artificially contaminated samples for microbiological analysis were used, respectively. Honey samples were infected with 10^6 spores/g.

- Physico-chemical analysis: colour (optical comparative method), moisture (refractometric method), pH, acidity (titrimetric method), glucose and fructose content (chromatographic method), hydroxymethylfurfural (HMF - chromatographic method), enzyme activities (diastase, α and β -glucosidase, glucoso-oxidase), organic acids content (formic acid: enzymatic method; oxalic acid: chromatographic method) (Bogdanov *et al.*, 1997) were considered.
- Microbial analysis: AFB spores were detected by culture (MYPGP agar, 37°C for 4-5 days) using a positive control (DMS 7030). Further identification of *P. larvae* colonies was carried out under microscope after Gram stain and using biochemical analysis (catalase and nitrate test).
- **Beeswax samples.** 14 beeswax samples (30 g/each) were tested for physico-chemical characteristics. Physico-chemical analysis: melting point, acid value, ester value, ratio number, free alcohols, free acids, hydrocarbons, esters (chromatographic method) were considered.

One-way and two-ways analysis of variance (ANOVA) were carried out to evaluate the effects of radiation treatment on physico-chemical parameters of beeswax and honey samples. When F-test value was significant, HSD-Tukey tests were used to detect differences between means. The Pearson correlation coefficient (r-value) was used to evaluate the correlation between parameters variation and radiation dose.

RESULTS

HIVE EQUIPMENT

None of the irradiated samples was positive to *P. larvae* irrespective of the radiation level applied.

HONEY SAMPLES

Physico-chemical analysis

The main physical modifications observed in honey were: bubbles formation, leakage of honey out of boxes, foamy scum on the surface, some cloudiness and light browning of *Robinia* honey samples. A decrease of viscosity and a breaking of glucose crystals were also observed. In fact, honeys irradiated at 25 kGray did not crystallize after treatment.

Concerning chemical parameters, the effect of radiation was significant ($F = 12.5$, $df = 33$, 6.6 $p < 0.001$). This effect was different in relation to type of honey ($F = 4.2$, $df = 66$, 16.2 , $p < 0.001$) and to type of parameters analysed. In particular, a decrease of enzyme activity resulted good correlated to the increase of radiation dose (diastase: $r =$, $t =$, $p =$; α -glucosidase: $r =$, β -glucosidase = , glucoso-oxidase =). Among the enzymes analysed, diastase activity resulted more sensible than the other ones and a significant decrease (on average 10%) was observed

already after a radiation treatment at 10 kGray dose *Robinia* and honeydew (Figure 1). Formic and oxalic acids are some of the radiolytic products of carbohydrates. In particular, a mean increase of 26.2% in formic acid after 10 kGray irradiation was registered. This increase was not significant and within the range of natural content.

Microbial analysis

After irradiation at all levels AFB-spores lost their infectivity. In fact, all the samples analysed were negative on culture.

BEESWAX SAMPLES

Physico-chemical analysis

In general, the effect of gamma radiation on beeswax samples was not significant ($F = 1.0$, $df = 12$, 2.9 , $p = ns$). A slight increase of melting point resulted proportional to radiation level, but this difference was not significant.

DISCUSSION

According to the literature (Gochnauer and Hamilton, 1970; Hornitzky and Willis, 1983), a 10 kGray dose radiation treatment provides the inhibition of AFB-spore germination in scales and in honey. Furthermore, at this level no particular changes in beeswax composition were observed. According to Katznelson and Robb (1962) and Wooton *et al.* (1985), the main modifications observed in honey were of physical changes. Among chemical properties, enzyme activity decreased with the increase of radiation level and, diastase activity was reduced already after a 10 kGray radiation treatment. The HMF content before and after radiation treatment did not change as demonstrated by Wooton *et al.* (1985), according also to its low content in the investigated samples.

In conclusion, gamma radiation treatment at 10 kGray could really be of additional value to beekeepers as routine sanitating treatment of beekeeping equipment. A large scale application of this treatment is then recommended.

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Figure 1: Diastase activity determination before and after radiation treatment at 10, 15 and 25 kGray of three different honeys (*Robinia*, Honeydew, Multifloral).

