# PROLIFERATION OF PATHOGENIC BACTERIA IN THE NEST OF THE *APIS MELLIFERA* FOLLOWING ATTACK BY *VARROA JACOBSONI* OUD

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*Varroa* is known to be present to a varying degree all over the Region of Tuscany.

The reproduction of the mite *(Varroa jacobsoni* Oud) inside colonies of *Apis mellifera* is more or less favoured by particular environmental conditions, and it finds different possibilities of development in the various ecotypes of bees present in the territory (Pinzauti et al., 1987).

During autumn 1985 and February-March 1986, the Chair of Apiculture of the Agricultural Entomology Section of the Department of Cultivation and Defence of Wooden Species of the University of Pisa received several reports from beekeepers of the provinces of Pisa and Legorn about bees abandoning the hive in spite of the presence in the honeycombs of large quantities of honey.

At the same time, other pathological cases, reported and analyzed visually, appeared to be related to forms of foulbrood, which could only be diagnosed by means of examinations based on laboratory cultures.

In the former case, the reason for the anomalous behaviour of the family is likely to be a wide-scale attack of *varroa,* especially at the end of the year, when all the mites present install themselves on adult bees as a replacement for the brood; in the latter case, however, a summary analysis of the brood present on the honeycombs led the analyst to diagnose a form of European foulbrood, in view of the various reasons for excluding the presence of American foulbrood, as well as the anomalous position assumed by several larvae in the cells without operculum.

Closer examination of the brood, both with and without operculum, revealed in all cases the presence of several varroae, both in "normal" larvae and pupae and in those close to putrefaction.

In collaboration with the University of Pisa, bacteriological examinations were carried out at the Operations Unit of Biotoxicology of the Local Sanitary Unit n. 13 on certain frames whose brood had been affected, in order to determine the etiology.

## Materials and methods

The larvae affected by the bacterial infection were removed using sterilised equipment, and homogenised in a mortar, using peptonised water. Samples of cell opercula and pollen

remains situated at the bottom of the cells themselves were similarly taken and treated.

The homogenates were inoculated on to selective and elective liquid and solid substrat ordinarily used for the study of polymicrobial samples likely to contain germs that are particularly demanding, with an aerobic-anaerobic metabolism.

The entities insolated were subsequently subjected to microscopic and biochemical investigation in order to define their taxonomy, in accordance with Bergey's Manual of Determinative Bacteriology (8th ed.).

Definite biochemical identification of the species was achieved using the multiple systems commonly used in bacteriology, produced by Ayerst and Roche, and known as: A.P.I. 2OE, A.P.I.N.E., 50 CH-MB, A.P.I. STAPH, A.P.I.-AN, OXI FERM TUBE, MYCOTUBE, and ENTEROTUBE II.

Tests were also made for ubiquitarian germs which particularly interest human pathology and zootechnics, such as *Yersinia enterocolitica* and *Campylobacter jejuni.*

## Results and Discussion

The multiplicity of microbial species found confirmed the results of the objective examination of the brood, which excluded American foulbrood, indicating rather the European kind. However, no trace was found of certain microorganisms which can be detected with a certain regularity in larvae affected by European foulbrood, according to recent publications (Bailey, 1981). In particular, no microbial entity was identified, either microscopically or in culture, as melissococcus pluton (white).

The concurrent, large-scale presence of *Varroa jacobsoni* led us to diagnose different foulbrood conditions sustained by occasional pathogenous microorganisms favoured by a decline in the organic defences of the bees which also influences the state of health of the brood.

Similar considerations can be found in human microbiology: saprophyte bacterial strains frequently become virulent as a result of prolonged antibiotic treatment or alterations in the conditions of cell or humoral defence. These localised infections, which are generally resistant to antibiotic treatments, are frequently observed. The cause is believed to lie with microbial agents coming from the environment, which are present on skin surfaces and mucosae (Micrococci, Staphylococci) or are a part of the normal intestinal bacterial flora (*Proteus, Escherichia coli, Enterobacter,* etc.) in bee pathologies has not been clearly explained, some authors would include these species in the group of microorganisms that are responsible for forms of septicaemia (Spina, 1971).

Besides causing the decomposi-

tion of dead bees, the *Serratia* kinds apper to be able to provoke malignant forms of septicaemia (Otte, 1967).

*Bacillus thuringensis* is notoriously able to cause the death of some insect larvae when contamination is massive.

Yeasts belonging to the *Candida, Torulopsis* and *Rhodotorula* kinds have repeatedly been isolated from larvae struck by European foulbrood (Kamburov and Hajsig, 1963).

The *Campylobacter* and *Yersinia* kinds were not detected.

As regards the tests with aromatic essences, the sensitivity of some of the microbial strains isolated was tested by means of the aromatogram technique (Belaiche, 1979), using essential oils that possess a known antimicrobial activity.

Essential oils are largely used in natural medicine in view of their ability to act as antiseptic agents and as compounds that correct the humoral substratum and stimulate the immune system. At low doses, they maintain all their antimicrobial activity, while not being very toxic for animals. They have recently been indicated as powerful tools in the fight against parassitosis and bee infections (Robaux, 1986).

In order to obtain pure essential oils for our tests, of a known origin, and with a well-defined composition, their extraction was performed from plants in our Institute, using the steam stream distillation technique.

Table 1 shows the results obtained.

*Table 1*

**Antimicrobial activity of essential oils of different species of plants**

| Essential oil | Some of isolated microbial strains | | | |
|---|---|---|---|---|
| | *Candida kruzei* | *Enterobacter agglomerans* | *Serratia rubideae* | *Bacillus thuringensis* |
| 1 | 2 | 3 | 4 | 5 |
| *Allium cepa* L. *Liliaceae* | R | R | R | SS |
| *Allium sativum* L. *Liliaceae* | S | S | R | SS |
| *Artemisia dracunculus* L. *Compositae* | PS | R | R | PS |
| *Carum carvi* L. *Ombrelliferae* | PS | PS | PS | PS |
| *Cimbopogon citratus* Stapf. *Graminaceae* | R | PS | S | SS |
| *Cinnamomum ceylanicum* B. *Lauraceae* | R | SS | S | S |
| *Corandrum sativum* L. | | | | |

*(continued table 1)*

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| *Ombrelliferae* | PS | S | PS | S |
| *Citrus aurantium* var. *dulcis* R. *Rutaceae* | PS | S | S | PS |
| *Citrus limonum* R. *Rutaceae* | SS | R | PS | S |
| *Cupressus sempervirens* L. *Coniferae* | S | PS | PS | SS |
| *Eucaliptus globulus* Lab. *Mirtaceae* | *SS* | *S* | *S* | *SS* |
| *Eugenia caryophillata* Thunb. *Mirtaceae* | SS | PS | PS | S |
| *Hysopus officinalis* L. *Labiateae* | PS | PS | R | PS |
| *Juniperus communis* L. *Coniferae* | R | R | R | R |
| *Lavandula offinalis* Chaix. *Labiateae* | PS | PS | PS | S |
| *Melaleuca viridifolia* Gaertn. *Mirtaceae* | S | S | PS | SS |
| *Mentha piperita* L. *Labiateae* | PS | S | PS | PS |
| *Ocymum basilicum* L. *Labiateae* | S | PS | R | S |
| *Origanum majorama* var. *hortensis* M. | PS | S | PS | PS |
| *Origanum vulgare* L. *Labiateae* | PS | SS | PS | SS |
| *Pelargonium odorantissimum* L. *Geraniac* | SS | R | R | PS |
| *Pinus sylvestris* L. *Coniferae* | S | R | PS | S |
| *Rosmarinus officinalis* L. *Labiateae* | PS | PS | R | SS |
| *Salvia officinalis* L. *Labiateae* | PS | PS | R | PS |
| *Santalum album, spicatum* L. *Jantalaceae* | R | R | R | PS |
| *Satureja ortensis* L. *Labiateae* | PS | R | R | SS |
| *Thuja occidentalis* L. *Cupressaceae* | PS | PS | PS | S |
| *Tymus vulgaris* L. *Labiateae* | SS | R | R | SS |
| *Terebenthinae* L. *Coniferae* | R | R | PS | PS |

The abbreviations R, PS and SS stand for the size of the halo of bacterial inhibition produced by the relative essential oil, measured on inoculated Petri's capsules; the lowest degree of sensitivity refers to haloes with a diameter of less than 10 mm, the medium level to haloes between 10 and 20 mm, and the highest sensitivity to haloes between 20 to 30 mm and more than 30 mm.

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