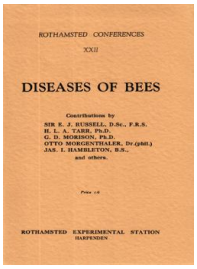
ROTHAMSTED RESEARCH

Diseases of Bees

Full Table of Content



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H. L . A. Tarr (1937) *Brood Diseases in England : the Results of a Three Year Investigation ;* Diseases Of Bees, pp 7 - 16 **- DOI: https://doi.org/10.23637/ERADOC-1-187**

BROOD DISEASES IN ENGLAND: THE RESULTS OF A THREE-YEAR INVESTIGATION

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In the time available it will only be possible to summarize briefly the results which have been obtained since the inception of the brood disease research scheme at Rothamsted. In doing this it will be necessary to assume some knowledge of the common characteristics of the various brood diseases: so much has been said and written about them recently that this demand would not appear unfair. The fact that there is indeed a multiplicity of brood diseases in England must be emphasised. Statements to the contrary have been and are being made by individuals who occupy prominent positions in beekeeping in this country: such statements are definitely erroneous. The fact that there is a multiplicity of brood diseases is of considerable importance when one has to consider their treatment.

Since May, 1934, two hundred and five samples of diseased brood have been sent to the laboratory and the following diagnoses have been made: American foul brood, 104; Addled brood, 58; European foul brood, 13; Chalk brood, 11; American foul brood and Addled brood, 1; American foul brood and Chalk brood, 1; chilled or neglected brood, 4; spray poisoning, 1; Sac brood, 4; drone laying queen and decomposing brood, 5; and drone laying queen and Chalk brood, 3. Though it cannot be stated that these figures denote the actual proportion of brood diseases in England, they certainly are of value in that they give some indication of their distribution, the proportion being relatively constant from year to year. It is practically certain that the distribution of the different diseases could not have been foretold prior to the commencement of the investigation. So far most of the time has been devoted to experiments designed to determine the causes of the three most prevalent brood diseases, for without this knowledge it would be difficult to devise treatment. The results of experiments relating to the different diseases will be discussed separately.

https://doi.org/10.23637/ERADOC-1-187

American foul brood

The examination of numerous samples has led to a verification of the fact that the spores of *Bacillus larvae* are almost invariably present in apparently pure culture in the ropy remains and scales of larvae dead of this disease, and further that this organism causes the disease. A number of experiments have been carried out in order to determine the relationship of *B. larvae* to the cause of American foul brood. It has been found that disease is caused by feeding decaying larvae, dead of American foul brood, to bees of healthy nuclei even when the material is suspended in water and heated for twenty minutes at 85°C (185° F), the spores of the causal organism resisting this temperature readily. Heating under steam pressure at a high temperature killed the spores of *B. larvae*, and material so treated was no longer capable of initiating disease. After considerable trouble a culture medium upon which *B. larvae* grew and sporulated readily was evolved : most media upon which this organism grows readily will not support spore formation. Suspensions of the vegetative cells and of the spores of *B. larvae* were prepared upon this medium and were employed in a series of infection experiments.

Vegetative cells of *B. larvae* when introduced into healthy nuclei by feeding the bees, feeding the larvae directly, or spraying the bacteria over developing brood, have in no experiment so far produced disease. Thus in two different experiments, approximately 170,000 million and 80,000 million vegetative cells prepared on the same medium as that employed for obtaining spores, were sprayed over eggs and developing larvae of healthy nuclei and no disease developed. Toumanoff, working in France, obtained similar results. With spores of *B. larvae*, obtained from pure cultures of the organism, American foul brood was readily initiated, providing a fairly large dose (mass inoculum) was employed. It was also found that a very much smaller inoculum of spores was effective in producing the disease, when the developing brood of the nucleus was sprayed directly with them than when they were fed to the bees in syrup. In one series of experiments, in which the same spore suspension was employed throughout, nuclei in which the brood was sprayed with approximately 620 million or 62 million spores soon developed American foul brood, while the disease did not develop in a nucleus receiving only approximately 6.2 million spores. When the spores were fed to the bees in syrup instead of being sprayed over developing brood, disease resulted in nuclei receiving approximately 62,000 million or 6,200 million spores, but not in those receiving approximately 620 million or 62 million spores. Sturtevant, working in the United States, found that a colony of bees would not develop American foul brood unless it received at least 50 million spores of *B. larvae* fed to the bees in 1 litre of syrup, the spores used in his experiments being derived from scales of larvae dead of the disease. The above work confirms, in general, his results. The fact that the

https://doi.org/10.23637/ERADOC-1-187

limiting infective dose in Sturtevant's experiments was considerably smaller than that used in the above described experiments may be because *B. larvae* rapidly loses virulence following cultivation on laboratory media. It seems highly probable that a few resistant endospores become established in the guts of very young larvae, and that once established they resist the digestive processes until conditions which favour their development arise, while the less resistant vegetative cells of the organism are rapidly killed under identical conditions.

It is of interest that, in these experiments, relatively large doses of vegetative cells of *B. larvae* would not cause American foul brood to develop. If a method of keeping this organism in the vegetative stage could be devised, then the control of the disease in infected colonies might be simplified. Unfortunately the possibility of doing this seems rather remote.

The results obtained have shown that American foul brood is the most prevalent brood disease in England, that it is a distinct disease caused by a resistant spore forming bacillus, and that a mass inoculum of spores of the organism is required to initiate the disease. So far no attempts have been made to study methods of controlling this disease. The very nature of the disease makes the possibility of obtaining a simple chemical remedy an extremely doubtful one, and it is not proposed to encourage any false hopes in this direction. Practical measures of control are known and the value of these under the conditions which pertain to this country must be determined.

Addled brood

The fact that this complaint should occupy such a prominent position with reference to the total number of brood diseases was not foreseen when the investigation commenced. Should the treatment of this very prevalent disease be as simple as is indicated by preliminary experiments we may well be pleased that so much of the disease is of this type and not so-called " foul brood." Before discussing the results obtained in practical experiments it is essential that brief reference be made to the somewhat scanty pertinent literature.

Throughout the past few decades numerous references have been made in the German literature to " Eitaubheit," a disease of bees in which apparently normal, fertile queens lay eggs which never develop. There is no adequate equivalent in the English language for this name and it is best expressed as Addled egg disease, the eggs being known as " Addled eggs " (Taube Eier). References to the condition in which brood dies at some stage prior to reaching maturity have been far less numerous in comparison. As far as I have been able to ascertain the late Dr. Leuenberger was the first to describe Addled brood though he did not actually employ this term. In one case of Eitaubheit he observed that a small

https://doi.org/10.23637/ERADOC-1-187

number of eggs succeeded in hatching, but that the larvae which developed from them, a few of which were sealed over by the bees, died before reaching maturity. This seems to have been a mixed case of Addled eggs and Addled brood, the queen being responsible for the condition. In 1925 Anderson described cases of Addled brood in Scotland. These cases were marked by the fact that practically all the sealed brood died just prior to the time of emergence, but occasionally addled eggs (eggs which did not hatch) were noticed, and, of the bees which did succeed in developing, many could not fly. The disease could be produced in healthy colonies by introducing queens from affected stocks to them, while requeening affected stocks with normal queens always cleared up the disease. Moreaux, working in France, has also recently described a case of Addled brood. His paper appeared at about the same time as the 1935 report on brood diseases was issued from this Station. In the last named article the appearance of larvae in cases of Addled brood (" Uncertain ") was described in some detail. It appears that the numerous cases of diseased brood received at this Station in which at first no accurate diagnosis could be made, and which were temporarily designated " Uncertain," were, in reality, cases of Addled brood. Unfortunately there is not time to describe in detail the appearance of larvae dead of this disease. Normally the pupae or prepupae are attacked, the appearance of the dead brood being similar to that noticed in larvae which have died and undergone autolysis. Usually these larvae are almost, or quite, sterile bacteriologically. The results of a series of experiments which have been carried out in order to ascertain the cause of the complaint leave no doubt that it is, in effect, Addled brood. There is every indication that the complaint can vary greatly in severity. Thus in some colonies relatively few Addled pupae are seen, and such a colony may show few or no external symptoms of weakness. On the other hand some stocks may be badly affected, and become so weak that they store no surplus honey and may even fail to resist the winter. It seems fairly certain that the cases investigated by Anderson merely represented very severe Addled brood in which very advanced pupae were those chiefly affected.

In three years, fifty-eight samples of Addled brood have been sent in, representing about 28 per cent. of all the samples received. Preliminary experiments showed that the disease, unlike American and European foul brood, is not contagious : combs containing an abundance of affected pupae when placed in healthy nuclei never caused disease. During the past season queens taken from affected stocks have been obtained from certain beekeepers, and several of these have been successfully introduced to queenless nuclei. In every case in which such a queen was accepted, the nucleus concerned soon showed signs of Addled brood ; a certain proportion of the sealed brood produced by the queen, dying before reaching maturity. When the affected queen was removed from

https://doi.org/10.23637/ERADOC-1-187

such a nucleus normal sealed brood soon appeared, following the introduction of a healthy queen. It seems that the disease is not necessarily one of old queens, for queens mated in 1936 have been found to produce Addled brood. One experiment has shown that drone as well as worker pupae are affected; this might be taken to indicate that the queen is directly responsible for the trouble and that the drone plays no part. The fact that eggs and very young larvae, when inserted in an affected colony, have been observed to develop normally, supports the idea that the queen causes the malady and that it is not due to lack of attention of the larvae by the nurse bees.

Though the superficial cause of the disease is a defective queen, the fundamental cause remains to be determined. The defect may be hereditary, the queen possessing some "lethal factor," or the queen may suffer from some infectious disease or from some abstruse pathological abnormality. This remains to be determined. Since further experiments on the control of this disease are needed, it is hoped that beekeepers who experience the complaint will try requeening affected stocks and will notify this Station of the result. The importance of continued investigation can readily be foreseen, especially in view of the fact that the queen breeder must at all costs eradicate the disease from his apiaries.

European foul brood

At present it appears that this disease is not widespread in England, but the fact that it does occur, that it is highly contagious, that it causes more trouble than American foul brood in Switzerland, and that its cause has been in doubt has made it advisable to investigate it thoroughly. If more is known about it, then it will be easier to employ measures to prevent its spread should it again show signs of increasing.

Larvae affected with European foul brood, unlike those dead of American foul brood, exhibit a very varied bacterial flora, and this fact has greatly complicated the determination of the cause of the disease. The remarkable confusion which has existed with reference to its etiology, has been referred to in a number of publications. The results of preliminary experiments carried out here, led to the suggestion that European foul brood might not be a single disease, but that it was, perhaps, a mixed bacterial infection of the brood of weak stocks of bees. Further experiments carried out during the past season have shown that this hypothesis was erroneous, and that the disease is, in fact, a single one in which various modifications may occur.

White (1912-1920) working at the United States Department of Agriculture, concluded, on the basis of a large number of experiments, that European foul brood was a single disease caused by a lanceolate-shaped coccus organism which he termed *Bacillus pluton*. He was unable to cultivate this organism on any laboratory

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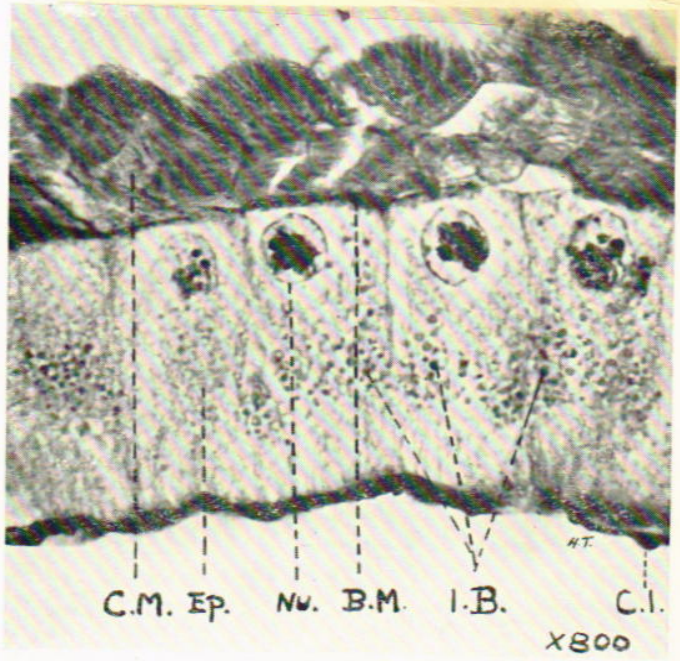

Fig. 1. Photomicrograph (×800) of a portion of a transverse section of the anterior end of the small intestine of a worker bee suffering from Bee Paralysis. The section was stained with safranin and methyl violet. B.M., basement membrane; C.I., chitinous intima lining the cavity of the small intestine; C.M., circular or transverse muscle fibre; Ep., epithelial cell with its nucleus Nu.; I.B., inclusion bodies which seem to be found only in bees suffering from Bee Paralysis.

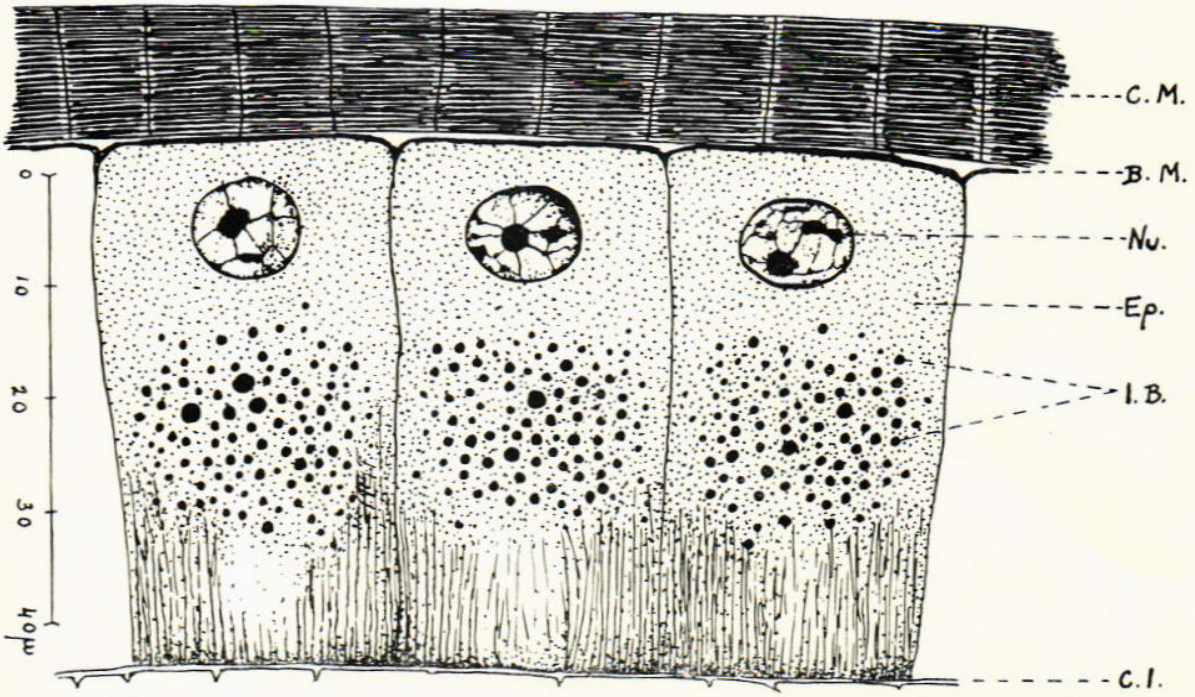

Fig. 2. Drawing made from a similar section. The legend is the same.

https://doi.org/10.23637/ERADOC-1-187

bees taken from brood combs containing large numbers of larvae sick or dead of European foul brood have quantities of bacteria in their rectal ampullae. Among these the secondary invaders found in European foul brood are usually most prominent, especially *B. alvei* and small rod-shaped bacteria, but *B. pluton* organisms are frequently present. European foul brood has been produced in a healthy nucleus by spraying an aqueous suspension of the gut contents of six such bees recently taken from an infected colony over the developing brood, but it is not known how long the parasite will remain alive in the gut of the bee. It is extremely doubtful if *B. pluton* multiplies in the digestive tract of the bee. All attempts to demonstrate *B. pluton* in the pharyngeal, mandibular or salivary glands of nurse or house-cleaning bees taken from infected stocks, either microscopically or by means of infection experiments, have failed. So far no evidence has been obtained which indicates that this organism multiplies elsewhere than in the gut of the young larva.

Queens from infected stocks have in no instance caused disease when introduced into healthy queenless nuclei. In these experiments the queen and 12 young worker bees were removed from the affected stock and were caged from 1-2 days with candy as the sole source of food prior to introduction. These results verify those obtained by Morgenthaler and his associates working in Switzerland.

European foul brood has been induced in healthy nuclei early in the brood rearing season by suspending in them combs containing large numbers of decomposing larvae artificially infected by feeding them pure cultures of *S. apis* or *B. alvei* and subsequently starving them for four days at hive temperature. These results have been obtained in each of two consecutive seasons. It has been found that when the disease is initiated in this manner it never appears as soon as in nuclei infected directly with *B. pluton* cells taken from the gut of a young infected larva, especially when this organism is sprayed directly on the eggs and young larvae. There seems to be a definite lag period, usually of about three weeks, during which no infected larvae are seen. It might be inferred from these experiments that *B. pluton* normally exists in colonies of bees waiting for suitable conditions to multiply, and that these conditions can be induced by putting into the colony very large numbers of decomposing larvae which have been artificially infected with secondary invaders found in European foul brood. This is only a suggestion which must be verified by further experiments. So far all attempts to induce European foul brood in healthy nuclei by "artificial weakening" (removing bees or sealed brood and giving a surplus of eggs and young larvae) have failed, but these experiments were not carried out early in the brood-rearing season. Normally European foul brood can only be induced readily in the early part of the brood-rearing season, and, unlike American foul brood, disappears, or tends to disappear, toward the close of the brood rearing.

https://doi.org/10.23637/ERADOC-1-187 *pp 8*

A thorough study of the control methods employed in the case of European foul brood must be made. In two cases in which swarms from colonies affected with European foul brood were hived on fresh foundation in clean hives the disease did not reappear. In another case in which a swarm was hived on drawn comb the disease soon broke out again. It is not improbable that the shaking method will prove of value in eradicating European foul brood in very obstinate cases of the disease which have failed to respond to the usual requeening treatment.

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