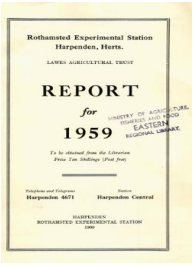
ROTHAMSTED RESEARCH

Report for 1959

Full Table of Content



Infectious Diseases of the Honeybee

L. Bailey

L. Bailey (1960) *Infectious Diseases of the Honeybee ;* Report For 1959, pp 204 - 215 **- DOI: https://doi.org/10.23637/ERADOC-1-92**

INFECTIOUS DISEASES OF THE HONEYBEE

By

L. Bailey

Research at Rothamsted on infectious and other diseases of the honeybee began in 1934, and was supported by a yearly grant of £250 for the first 3 years from the British Beekeepers Association and an equal sum from the Agricultural Research Council, together with contributions from private individuals. The grants were primarily for work on diseases of honeybee larvae—brood diseases—incidence of which in England and Wales was causing concern. This review is of the research done at Rothamsted since then on infectious diseases of honeybees, and of relevant work elsewhere. There are infections of honeybees other than those mentioned below, but they are of less significance or incidence in England and Wales, and consequently have not been specially studied at Rothamsted. H. L. A. Tarr began the research: his work was mainly on the natural history of Bacillus larvae, established by White (1907) as the cause of American foul brood disease (AFB), and on the etiology of European foul brood disease (EFB).

American four brood disease

Sturtevant (1924), finding that reducing sugars, particularly in concentrations over 2 or 3%, inhibited germination of spores and vegetative growth *in vitro* of *Bacillus larvae*, suggested that this is why only sealed larvae, which have consumed all their food and the sugar it contains, become diseased. Tarr (1938a), however, found that spores germinated in a liquid medium of minced chicken embryo with concentrations of reducing sugars up to 12·5%. Furthermore, even small numbers of spores (100–140) sometimes would germinate in his medium. Lochhead (1933) also found that a few spores germinated below the surface of semi-solid media of entirely different constitution to Tarr's, and it seems possible, therefore, that spores germinate best in a critical, reduced-oxygen tension. Previously, Sturtevant (1932) found only large inocula of spores germinated *in vitro* and thought that this may explain why small doses fail to cause disease in bee colonies: a few larvae infected when 4–5 days old died in his experiments, but only when inoculated with massive doses of about 10 million spores each.

Tarr (1937a) showed that spores were necessary to infect larvae: vegetative cells, even in massive doses, did not infect. Unlike Sturtevant, however, he could not cause AFB by inoculating the food of 4–5-day-old larvae. He infected larvae more successfully by spraying them with spores than by feeding their colonies spores in syrup, and he noted young larvae seemed more susceptible. Subsequently Woodrow (1942) found one spore enough to cause disease provided the infected larva was younger than 1 day old: larvae

https://doi.org/10.23637/ERADOC-1-92

older than 2 days were immune. On this basis it is difficult to explain why only sealed larvae die of disease, particularly as spores germinate within 24 hours after entering the larval mid-gut (Woodrow & Holst, 1942). Furthermore, the vegetative rods do not grow much in unsealed larvae (Maassen, 1908), and may even diminish in numbers shortly after larvae are sealed (Holst, 1946). *Bacillus larvae* may not grow much in the larval intestine, as this is probably too anaerobic, or bacterial growth quickly makes it so (see EFB section below), particularly perhaps in the most rapid growth phase of the larva which starts on the 3rd day after hatching (Nelson, 1924). Larvae may ultimately be killed during a critical phase of pupation by the toxic enzymes that Patel & Gochnauer (1959) found in remains of larvae dead of AFB, and which may be liberated by *B. larvae* as it grows. The relatively few bacteria in the larvae then could invade the dead tissues, proliferate and sporulate.

Shortly after Haseman & Childers (1944) found that sulphonamide drugs effectively suppressed signs of AFB, experiments were made at Rothamsted to test the value of such treatment. Milne (1947) summarised his investigations and confirmed the effectiveness of sulphonamides in allaying disease, but emphasised that treatment was impermanent, because dormant spores of *Bacillus larvae*, now known to stay viable for at least 33 years (Haseman, 1959), can develop once the drug is exhausted. In view of the lessening of the disease in England and Wales since the implementation of the Foul Brood Disease of Bees Order of 1942, which enforces destruction of known diseased colonies, use of sulphonamides was not considered advisable, and work on chemotherapy of brood diseases was suspended at Rothamsted. Work continued abroad, however, principally in Canada and the United States: oxytetracycline (Terramycin) was found by Katznelson & Jamieson (1952b) to be an effective antibiotic, but it has the same limitations as sulphonamides. There is still controversy, even in countries where use of drugs is permissible, about the desirability of their use in preference to destroying diseased colonies.

European foul brood disease

Tarr began investigations at Rothamsted on EFB when there was considerable confusion, not only about the etiology of the disease, but even about its existence. He worked first on *Bacillus alvei*, a bacterium commonly present in remains of larvae that have died of the disease and was, at the time, usually considered the cause. Burnside & Foster (1935) described *Bacillus para-alvei* as an organism similar to *B. alvei* and causing a disease similar to EFB. Tarr (1936a) found that *B. alvei* and *B. para-alvei* were biochemically indistinguishable; their differences, which were slight, were solely morphological. Davis & Tarr (1936) also found *Streptococcus apis* (another organism often present in diseased larvae) was indistinguishable from *Streptococcus liquefaciens* or *Streptococcus glycerinaceus* (all now classified as *Streptococcus faecalis*: see Bergey's *Manual of Determinative Bacteriology*, 6th ed.). This was apparently independent of Hucker (1932), who had already identified *A. apis* as *S. liquefaciens* by cultural and serological tests. At first Tarr (1936b) thought *A. apis* or *B. alvei* were able to cause EFB: he

https://doi.org/10.23637/ERADOC-1-92

found that disease sometimes developed after larvae had their food inoculated with pure cultures of either organism and were incubated without nurse bees for 4 days at 35° before being returned to their parent colony. It seems likely that EFB was endemic in the experimental colonies he used, and this may have made results unreliable, but it is also possible that signs similar to those of EFB were induced in the inoculated larvae whose resistance was lowered by starvation (see below). Later, Tarr (1937b) realised, as had White (1912), that another organism always seemed to occur in diseased larvae, particularly in the early stages of their infection. It was hardly distinguishable morphologically from *S. apis*, but its presence was suspected when attempts to make cultures *in vitro* from larvae, apparently containing very large numbers of *S. apis*, failed. Tarr (1938b) attempted to cultivate the organism, which he now recognised as *Bacillus pluton* White, on a variety of media without success. However, he confirmed many observations of White (1912, 1920).

Thus Tarr usefully clarified the complicated bacteriology of EFB, and his eventual confirmation of White's original observations, which previously had not been accepted or had been considerably modified, encouraged continuation of work with the principal fundamental aim of cultivation, *in vitro*, of *Bacillus pluton*. Meanwhile Burri (1943) introduced further complications by claiming to identify *B. pluton* as a dissociant form of *Bacterium eurydice* White, another organism commonly plentiful in larvae with EFB. Miss E. Kops at Rothamsted could not verify Burris' observations (*Rep. Rothamst. exp. Sta.* for 1947) but found *B. eurydice* to be pleomorphic and in some conditions to resemble *B. pluton* morphologically. Miss Kops and Miss H. Finegan tested a wide variety of media and conditions for cultivation of *B. pluton*, but all were unsatisfactory. Later, attempts were renewed with the co-operation of Professor L. P. Garrod (St. Bartholomew's Hospital), and field trials with one of his isolates indicated the possibility that *B. pluton* could be cultivated as an anaerobe (*Rep. Rothamst. exp. Sta.* for 1954). A medium was eventually developed at Rothamsted which gave satisfactory growth of *B. pluton* in anaerobic conditions (Bailey, 1957a), and the first experiments made with pure cultures showed it to be the primary pathogen in EFB (Bailey, 1957b, 1957c). As the organism does not form spores, it was decided that *Streptococcus pluton* (White), suggested by Gubler (1954), was a more appropriate name. Apart from low oxygen tension, the major critical requirements of *S. pluton* for growth *in vitro* are a high ratio of potassium to sodium, unidentified constituents of certain yeast extracts, high inorganic phosphate concentration, and CO_2.

Unlike *Bacillus larvae* (the cause of AFB), *Streptococcus pluton* develops abundantly in the larval mid-gut, suggesting that the mid-gut is virtually anaerobic, which may explain the feeble development of *B. larvae* in growing larvae. *S. pluton* apparently weakens larvae directly by depriving them of food (Bailey, 1959a), which enables secondary invaders to develop abundantly and help kill the larvae. Typically, *Bacterium eurydice* is the first of these to develop, as it is commonly present in bee colonies, living normally, apparently harmlessly, in the anterior parts of the alimentary tract of adult

https://doi.org/10.23637/ERADOC-1-92

bees (*Reps. Rothamst. exp. Sta.* for 1957, 1958). Other secondary invaders, such as *Streptococcus faecalis*, which seems common in cases of EFB in continental Europe, but not now in Britain, are probably picked up from outside sources by foraging bees. *Bacillus alvei* is not always present, but tends to become established in endemically infected colonies: it seems to be the last of the secondary invaders, developing in larvae that have died (Bailey, 1959b).

Tests with sulphonamides against EFB were made at Rothamsted in 1946 and appeared ineffective. Various antibiotics, however, particularly streptomycin and oxytetracycline, were found by Katznelson *et al.* (1952) to be effective, and their use has become common in North America and Europe. Growth of *Streptococcus pluton* is completely inhibited *in vitro* by penicillin G (concentration 10^{-7}–10^{-9}); oxytetracyclin (10^{-5}–10^{-7}) and streptomycin (10^{-4}–10^{-6}) (*Rep. Rothamst. exp. Sta.* for 1958). Despite the great efficiency of penicillin G *in vitro*, it is ineffective *in vivo*, against both AFB and EFB, and probably because it is unstable, particularly at pH values about 4·0, such as occur in honey and larval food. Dormant cells of *S. pluton* are now known to remain viable for over a year, however, and they probably become well distributed within bee colonies from some infected larvae, which, nevertheless, survive. Such larvae void very many bacteria in their faeces before pupation (Bailey, 1959a). Thus, treatment with antibiotics has, fundamentally, the same disadvantages with EFB as with AFB—continued application of drugs is needed until dormant bacteria have been eliminated by consumption of contaminated food and cleaning of combs by adult bees, but there can be no certainty of when this has been achieved.

Acarine disease

After the mite, *Acarapis woodi* (Rennie), was discovered by Rennie *et al.* (1921), it quickly acquired the reputation of being very destructive to adult bees, because it was considered to cause the " Isle of Wight disease ", from which many colonies of honeybees were alleged to have died in the British Isles from 1906 until shortly after the time *A. woodi* was discovered. Accordingly, the limited research at first possible at Rothamsted on adult bee infections was aimed at improved remedial measures, particularly as surveys between 1941 and 1944 (Butler, 1945) showed 20% of colonies infected and widely distributed in England and Wales. It had already been shown elsewhere that careful application of the vapour from Frow's mixture (nitrobenzene, safrol and petrol), or from burning sulphur, killed mites with no apparent damage to the bees. Butler (1941) also found terpineol vapour effective in laboratory tests. Later, more extensive field and laboratory trials were made with a variety of fumigants, including newer acaricides applied as smokes and reported effective by continental workers (Bailey & Carlisle, 1956). Briefly, Frow's mixture was found effective when applied in cold autumn weather, but infested bees eventually died in winter earlier than is normal. Thus, although the chances of heavy infestations recurring next season can be decreased, even if not eliminated, treated colonies are more likely than uninfested ones to die in the late winter because their numbers are abnormally low. Newer

https://doi.org/10.23637/ERADOC-1-92

acaricides of the di-(*p*-chlorophenyl) methyl carbinol ("Dimite") type are convenient for spring or summer use and are more effective than sulphur fumes. Repeated applications are necessary, however, unless they are used in warm weather, but then the colonies may be damaged because the smoke over-excites the bees.

Acarapis woodi achieved its bad reputation without any quantitative observations or experimental evidence about the pathology and epidemiology of infestation. Accordingly, since 1951, records were kept at Rothamsted of infestation and mortality of colonies that were not treated, and these showed that infestations in endemically infested hives are usually suppressed naturally. The proportion of total colonies examined that was detectably infested was similar to that found in the national surveys made in 1941–44, but most were only lightly infested, and when colonies found infested at any one time were graded according to the degree of infestation the numbers in each group fell exponentially as the degree of infestation increased. Few colonies ever had more than 30% of their bees infested during any one season. Obvious damage to colonies was found only with these heavy infestations, and then only in late winter: the very few with more than 75% bees infested usually die about March (Bailey, 1958). The effect of infestation on the life of individuals is slight and difficult to detect in summer bees. Queenlessness and inactivity of colonies in poor seasons are principal factors causing increased infestations (Bailey & Lee, 1959).

Thus, heavy infestation is the consequence of events unfavourable to colony development: it may be more common after several poor seasons, when the bees are kept alive by artificial feeding. On this basis, the apparent close association of mite infestation with "Isle of Wight disease" (Rennie *et al.*, 1921) may be reinterpreted. This disease probably had no single cause; rather, the name included a wide variety of debilities with similar signs (Rennie, 1923). Heavy mite infestation may well have resulted from, not caused, these various ailments, which on some occasions at least, including those of Rennie's investigations, occurred in very poor seasons. From its known wide distribution, *Acarapis woodi* was probably endemic in honeybees before the days of the "Isle of Wight disease": it has been found in *Apis mellifera* in most parts of Europe, including Sardinia and Mallorca, in Russia and in South America. It also occurs in *Apis indica* in India (Sardar Singh, 1956) and in *Apis mellifera adansoni* in the Belgian Congo (Benoit, 1959). Its apparent absence from Scandinavia, North America, Australia and New Zealand (Jeffree, 1959) seems remarkable, therefore, and deserves study.

As heavy infestation by *Acarapis woodi* is usually symptomatic of a poor economy, treatment, however successful in killing mites, may not be expected to produce a striking response in colony development. The value, therefore, of treatments that are directed solely against mites needs careful consideration, particularly as present methods have some detrimental effect on bees.

Nosema disease

In a survey in 1941–44 (Butler, 1945) adult bees infected with *Nosema apis* Zander were detected in about 5% of colonies widely

https://doi.org/10.23637/ERADOC-1-92 *pp 6*

distributed in England and Wales in April and May, the period when an acute peak of infection occurs in endemically infected colonies. This annual epidemic had been reported from Switzerland (Morgenthaler, 1939) and was confirmed at Rothamsted (*Rep. Rothamst. exp. Sta.* for 1939/45). Widespread infection of colonies at Rothamsted was first noticed in 1946, and from 1947 to 1950 Hassanein studied the natural history and pathology of the infection. Earlier workers showed that heavy artificial infection in autumn killed colonies in winter (White, 1919; Morgenthaler, 1941); that infected queens soon become unable to produce eggs (Fyg, 1945) and that hypopharyngeal glands of infected bees become prematurely atrophied (Lotmar, 1936). Hassanein (1951, 1952a) confirmed the last two observations, and found that brood-rearing was lessened in naturally infected colonies in spring, presumably because infected bees produced less hypopharyngeal brood-food, and that infected bees began foraging earlier and had shorter lives than uninfected individuals (Hassanein, 1953). There was plenty of experimental evidence, therefore, that infection with *N. apis* was pathological—more so, to individual bees, apparently, than infestation with *Acarapis woodi*.

Endemically infected but otherwise normal colonies are rarely obviously affected, however; this follows from the extent to which infection is naturally suppressed in such colonies during the summer. Burnside and Revell (1948) considered that increased temperature of the cluster, as brood-rearing increased, could account for the suppression of infection, and they confirmed results of Lotmar (1943a) showing that temperatures over 35° suppressed development of the parasite in individual, caged bees. An alternative explanation was that transmission of parasites to newly emerging bees, which are free of infection, ceases in summer when bees fly freely and defaecate outside the hive, and this was tested by experiments at Rothamsted in which bees were transferred to uncontaminated combs in early summer (Bailey, 1955a, 1955b). This treatment usually lowered infection to undetectable levels with no recurrence the next year. It seemed reasonable to suppose, therefore, that infection normally persisted in summer as faecal contamination deposited on combs the previous winter, and the decline of infection in summer reflected fewer spores left on combs to infect new bees. Recent experiments (Bailey, 1959c) showed that artificially infected bees, introduced to endemically infected colonies in summer when infection was naturally diminishing, all developed similar numbers of spores to those in naturally infected bees in spring when natural infection was high. Thus, high temperature seems to be unimportant, and the amount of infection on combs seems fundamental to the natural history of infection.

Means of decontaminating combs were developed: the best found was fumigation, at normal temperatures, with fumes of formaldehyde for empty combs, or with vapour of acetic acid for combs with food in them. The effectiveness of acetic acid was confirmed by beekeeping institutes abroad (Gavrilov, 1957; Jordan, 1957; Lunder, 1957). At Rothamsted almost all the bees were transferred to such combs in early summer 1954, and the percentage of colonies with detectable infection fell from between 50 and 90 to 7 the following spring (*Rep. Rothamst. exp. Sta.* for 1955). Many

https://doi.org/10.23637/ERADOC-1-92

colonies were also treated with fumagillin (see below), which may have helped, but there was no significant difference between colonies that received both treatments and those only fumigated (Bailey 1955b). These experiments were made in a poor season when infection locally, and generally in England and Wales, rose from 10 or 20 to 35 or 40% of colonies (Min. of Agric., Fish. & Food, 1956). Absolute elimination of infection seems unlikely by the methods employed, but keeping levels of infection low would be acceptable provided this could be done cheaply. Fumigating spare combs only, before re-use, has so far proved inadequate, but at Rothamsted colonies are handled more often than in normal beekeeping, and the seasons since the trial have been very poor. Nevertheless, this simplified method has some value, because the numbers of potential parasites are decreased; the susceptibility of wax-moths, particularly their eggs, and *Streptococcus pluton* to the same treatment (*Reps. Rothamst. exp. Sta.* for 1955, 1958) gives it added value.

Direct evidence of the effect of manipulation on infection of colonies was obtained (*Rep. Rothamst. exp. Sta.* for 1958) when infection increased significantly in colonies inspected monthly in winter. A similar but smaller effect may be expected in summer, and the high levels of infection of package bees from the southern states of the United States (Reinhart, 1942; Farrar, 1947) may derive from the handling they undergo followed by their long journeys. Infected samples of bees received for diagnosis about April and May by the National Agricultural Advisory Service have risen from the 1941–44 level of about 5% to approximately 20% since about 1953. This may not be entirely attributable to poor weather: there is no doubt that transportation of bees, mainly to orchards in spring and to heather in autumn, is more frequent now than during and shortly after the war of 1939–46, and transportation seems an important cause of high levels of infection (Bailey 1955a). Commercial beekeepers have considerably higher infection in their colonies than have beekeepers in general (unpublished data, Min. of Agric., Fish. & Food) and probably transport their bees more.

After the discovery of the striking effect of fumagillin in suppressing infection by *Nosema apis* (Katznelson & Jamieson, 1952a) field trials were made with it at Rothamsted. Feeding fumagillin in autumn prevented or retarded development of infection the following winter (Bailey, 1953a, 1955b). Results were not wholly satisfactory: infection was apparently always checked or diminished by treatment, but absolute elimination seemed unlikely. Repeated autumn application may eventually succeed: spring treatment alleviates the immediate acute infection, but comb contamination is less likely to be decreased.

It is necessary to consider the value of possible treatments and the circumstances in which they are applied. The degree of infection in spring with *Nosema apis* reflects to some extent the degree to which other circumstances of honeybees were unfavourable the year before. Lotmar (1943b) found a significant positive correlation between wet summers and the degree of infection the next year, and the only striking fall of infection in England and Wales in recent years was after the unusually good season of 1955 (Min. of Agric., Fish. & Food, 1956, 1957, 1958). In poor seasons the natural

https://doi.org/10.23637/ERADOC-1-92

cleaning of combs is probably decreased, because brood rearing and nectar gathering is diminished and defaecation by infected bees in the hive may be more frequent than in good seasons. Colonies should not be disturbed in poor seasons, and drug treatment seems most useful at the end of such seasons. Comb changing would be most likely to eliminate infection in good seasons.

More fundamental studies of infection in individual bees show that the parasite is first established at the anterior and posterior ends of the ventriculus, usually mostly at the anterior end, with a region of minimal infection centrally (Bailey, 1955c). The last region has many cytoplasmic granules of calcium phosphate which disappear when infection penetrates the cells. Their presence may, therefore, initially inhibit growth and development of *Nosema apis*. Infection soon disappears from bees fed syrup containing fumagillin, leaving apparently normal cells except at the anterior end of the ventriculus, where infection persists even after continuous drug treatment for several weeks (Bailey, 1953b).

Amoeba disease

Malpighamoeba mellificae Prell was studied at Rothamsted first by Hassanein (1952b), who confirmed that an annual epidemic, similar in character to that of *Nosema apis*, occurs in adult bees of endemically infected colonies. This annual epidemic appears to have the same explanation as that of *Nosema apis*; transference of infected colonies to non-contaminated combs in early summer eliminated infection, and transference of combs from infected to uninfected colonies in autumn introduced infection which became epidemic the following spring (Bailey, 1957d). Cysts of *M. mellificae* on combs were killed as readily as spores of *N. apis* by vapour of acetic acid, but fumagillin did not affect the development of *M. mellificae* in infected bees (Bailey, 1955d).

The incidence of infection with *Malpighamoeba mellificae* in England and Wales was less than 1% in 1941–44 (Butler, 1945), but in recent years it has risen to about 3%. The rise is coincident with that of *Nosema apis* mentioned above, and seems likely to be for similar reasons. Infection is restricted to the south-east of England, however, particularly near London (Min. of Agric., Fish. & Food, 1956, 1957, 1958), which is strikingly similar to infection reported from Denmark, where most is near Copenhagen (Fredskild, 1955). The reason for this is not clear, but bees may be examined and disturbed more frequently in urban than rural regions. Much more disturbance seems necessary to maintain abnormally high levels of infection by *M. mellificae* than by *N. apis*, as the cyst stage of the former does not appear until 3 weeks after infection (Fyg, 1932; Hassanein, 1952b), so that in summer, infected bees will be foragers that are almost at the end of their lives before they become infective. Infective spores of *N. apis* form after 10 days or less—well within the life span of the bee in summer.

Paralysis

Results of experiments by Burnside (1933) made it appear that adult bees with " paralysis "—dark, greasy-looking, virtually hairless bees, with sprawled legs and wings—had an infectious disease

https://doi.org/10.23637/ERADOC-1-92

that was transmitted directly between them. Butler (1943) made similar laboratory tests with the same results, but at the same time he pointed out that a variety of diseases, some non-infectious, and various poisons may produce similar signs. Burnside (1945) made further controlled laboratory experiments which seemed to show that the infectious disease was caused by a filterable virus. About 2% of samples of bees sent to the National Agricultural Advisory Service annually from England and Wales are diagnosed as paralysis, but the proportion that are of the infectious variety is unknown.

Conclusions

The common infections of the adult honeybee are not principal factors limiting the survival and growth of endemically infected colonies that are otherwise normal. But they tend to reach serious proportions when such colonies suffer set-backs. The set-backs are sometimes adventitious, but they are often imposed or aggravated by beekeeping practices. The increasing knowledge of the natural histories of adult bee infections should help to mitigate these actions. An understanding of the striking annual epidemic of EFB, which usually occurs in endemically infected colonies, should produce similar advantages. More epidemiological studies of AFB may be helpful, even though natural control of this disease, once present, seems poor.

Devising prophylactic measures depends on fundamental knowledge of the natural history of the infection concerned. Direct therapy may often be improved and evaluated by such information. Accordingly, the accumulation of fundamental knowledge remains the primary purpose of research on diseases of the honeybee at Rothamsted.

References

Bailey, L. (1953a). The treatment of Nosema disease with fumagillin. *Bee World*, **34**, 136–137.

Bailey, L. (1953b). Effect of fumagillin upon *Nosema apis* Zander. *Nature, Lond.* **171**, 212 only.

Bailey, L. (1955a). The epidemiology and control of Nosema disease of the honeybee. *Ann. appl. Biol.* **43**, 379–389.

Bailey, L. (1955b). Results of field trials at Rothamsted of control methods for Nosema disease. *Bee World*, **36**, 121–125.

Bailey, L. (1955c). The infection of the ventriculus of the adult honeybee with *Nosema apis* Zander. *Parasitology*, **45**, 86–94.

Bailey, L. (1955d). Control of Amoeba disease by the fumigation of combs. *Bee World*, **36**, 162–163.

Bailey, L. (1957a). The isolation and cultural characteristics of *Streptococcus pluton* (*Bacillus pluton* White) and further observations on *Bacterium eurydice*. *J. gen. Microbiol.* **17**, 39–48.

Bailey, L. (1957b). European foul brood, a disease of the larval honeybee (*Apis mellifera L.*) caused by a combination of *Streptococcus pluton* (*Bacillus pluton* White) and *Bacterium eurydice* White. *Nature, Lond.* **180**, 1214–1215.

Bailey, L. (1957c). The cause of European foul brood. *Bee World*, **38**, 85–89.

Bailey, L. (1957d). Comb fumigation for Nosema disease. *Amer. Bee J.* **97**, 24–26.

Bailey, L. (1958). The epidemiology of the infestation of the honeybee, *Apis mellifera* L., by the mite *Acarapis woodi* (Rennie), and the mortality of infested bees. *Parasitology*, **48**, 493–506.

https://doi.org/10.23637/ERADOC-1-92

BAILEY, L. (1959a). An improved method for the isolation of *Streptococcus pluton* and observations on its distribution and ecology. *J. Insect. Path.* **1**, 80–85.

BAILEY, L. (1959b). Recent research on the natural history of European foul brood disease. *Bee World*, **40**, 66–70.

BAILEY, L. (1959c). The natural mechanism of suppression of *Nosema apis* Zander in enzootically infected colonies of honeybee (*Apis mellifera* Linnaeus). *J. Insect Path.* **1**, 347–350.

BAILEY, L. & CARLISLE, E. (1956). Test with Acaricides on *Acarapis woodi* (Rennie). *Bee World*, **37**, 85–94.

BAILEY, L. & LEE, D. C. (1959). The effect of infestation with *Acarapis woodi* (Rennie) on the mortality of honeybees. *J. Insect Path.* **1**, 15–24.

BENOIT, P. L. G. (1959). The occurrence of the Acarine mite, *Acarapis woodi*, in the honeybee in the Belgian Congo. *Bee World*, **40**, 156 only.

BURNSIDE, C. E. (1933). Preliminary observations on " paralysis " of honeybees. *J. econ. Ent.* **26**, 162–168.

BURNSIDE, C. E. (1945). The cause of paralysis of honeybees. *Amer. Bee J.* **85**, 354–355.

BURNSIDE, C. E. & FOSTER, R. E. (1935). Studies on the bacteria associated with parafoulbrood. *J. econ. Ent.* **28**, 578–584.

BURNSIDE, C. E. & REVELL, I. L. (1948). Observations on *Nosema* disease of honeybees. *J. econ. Ent.* **41**, 603–607.

BURRI, R. (1943). Weitere Beobachtungen über Formwandlungen beim Erreger der Sauerbrut der Bienen. *Beih. Schweiz. Bienenztg*, **1**, 209–260.

BUTLER, C. G. (1941). A possible new cure for acarine disease of honeybees. *Nature, Lond.* **148**, 86 only.

BUTLER, C. G. (1943). Bee paralysis. May sickness, etc. *Bee World*, **24**, 3–7.

BUTLER, C. G. (1945). The incidence and distribution of some diseases of the adult honeybee (*Apis mellifera* L.) in England and Wales. *Ann. appl. Biol.* **32**, 344–351.

DAVIS, J. G. & TARR, H. L. A. (1936). Relation of so-called *Streptococcus apis* to certain Lactic Acid Streptococci. *Nature, Lond.* **138**, 763 only.

FARRAR, C. L. (1947). Nosema losses in package bees as related to queen supersedure and honey yields. *J. econ. Ent.* **40**, 333–338.

FREDSKILD, B. (1955). Ambesygens udbredelse. *Tiddskr. Biavl.* **89**, 121–123.

FYG, W. (1932). Beobachtungen über die Amoeben-Infektion (" Cystenkrankheit ") der Malpigischen Gefässe bei der Honigbiene. *Schweiz. Bienenztg*, **55**, 1–17.

FYG, W. (1945). Der Einfluss der Nosema-Infektion auf die Eierstöcke der Bienenkonigin. *Schweiz. Bienenztg*, **68**, 67–72.

GAVRILOV, B. N. (1957). Primenenie uksusnov kislotȳ v bor'be s nozematozom pchel. *Pchelovodstvo*, **34**, 47–50.

GUBLER, M. U. (1954). Bakteriologische Untersuchungen über die gutartige Faulbrut der Honigbiene (*Apis mellifica* L.). *Schweiz Z. allg. Path.* **17**, 507–513.

HASEMAN, L. (1959). How long may spores of American foulbrood remain viable? *Proc. 1st Mtg. Amer. Comm. Bee Res. Ass.*

HASEMAN, L. & CHILDERS, L. F. (1944). Controlling American foulbrood with sulfa drugs. *Bull. Mo. agric. Exp. Sta.* **482**, 1–16.

HASSANEIN, M. H. (1951). Studies on the effect of infection with *Nosema apis* on the physiology of the queen honey bee. *Quart. J. Micr. Sci.* **92**, 225–231.

HASSANEIN, M. H. (1952a). The effects of infection with *Nosema apis* on the pharyngeal salivary glands of the worker honey bee. *Proc. R. ent. Soc. Lond.* (*A*), **27**, 22–27.

HASSANEIN, M. H. (1952b). Some studies on amoeba disease. *Bee World*, **33**, 109–112.

HASSANEIN, M. H. (1953). The influence of infection with *Nosema apis* on the activities and longevity of the worker honey bee. *Ann. appl. Biol.* **40**, 418–423.

HOLST, E. C. (1946). Newer knowledge of American foulbrood. *Glean. Bee Cult.* **74**, 138–139.

HUCKER, G. J. (1932). Studies on the Coccaciae. XVII. Agglutination as a means of differentiating the species of Streptococcus and Leuconastoc. *Tech. Bull. N.Y. St. agric. Exp. Sta.* No. 190.

JEFFREE, E. P. (1959). The world distribution of Acarine disease of honeybees and its probable dependence on meteorological factors. *Bee World*, **40**, 4–15.

JORDAN, R. (1957). Eissigsäure zur Bekämpfung der Wachsmotte vor allem aber zum Entkeimen nosemainfizierter Waben. *Bienenvater, Wien*, **78**, 163–169.

KATZNELSON, H., ARNOTT, J. H. & BLAND, S. E. (1952). Preliminary report on the treatment of European foulbrood of honeybees with antibiotics. *Sci. Agric.* **32**, 180–184.

KATZNELSON, H. & JAMIESON, C. A. (1952a). Control of Nosema disease of honeybees with fumagillin. *Science*, **115**, 70–71.

KATZNELSON, H. & JAMIESON, C. A. (1952b). Antibiotics and other chemotherapeutic agents in the control of bee diseases. *Sci. Agric.* **32**, 219–229.

LOCHHEAD, A. G. (1933). Semi-solid medium for the cultivation of *Bacillus larvae*. *Bee World*, **14**, 114–115.

LOTMAR, R. (1936). Nosema-Infektion und ihr einfluss auf die Entwicklung der Futtersaftdrüse. *Schweiz. Bienenztg*, **59**, 33–36.

LOTMAR, R. (1943a). Über den Einfluss der Temperatur auf der Parasiten *Nosema apis*. *Beih. Schweiz. Bienenztg*, **1**, 261–284.

LOTMAR, R. (1943b). Bestehen Beziehungen zwischen der Witterung und dem seuchen Auftreten der Fruhjahrschwindsucht? *Schweiz. Bienenztg*, **66**, 68—80.

LUNDER, R. (1957). Nosemaproblemet i nytt lys. *Nord. Bitidskr.* **9**, 107–114.

MAASSEN, A. (1908). Zur etiologie der sogenannten. Faulbrut der Honigbienen. *Arb. Biol. Anst., Berl.* **6**, 53–70.

MILNE, P. S. (1947). Sulphonamide treatment of American foul brood. *Agriculture, Lond.* **54**, 82–87.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1956, –57, –58). *Survey of Bee Health and Beekeeping in England and Wales*, (Bee Disease Advisory Committee).

MORGENTHALER, O. (1939). Die ansteckende Fruhjahrsschwindsucht (Nosema-Amöben-Infektion) der Bienen. *Schweiz. Bienenztg*, **62**, 154–162.

MORGENTHALER, O. (1941). Einwinterung und Nosema. *Schweiz. Bienenztg*, **64**, 401–404.

NELSON, J. A. (1924). Growth and feeding of honeybee larvae. *Dep. Bull. U.S. Dep. Agric.* no. 1222.

PATEL, N. G. & GOCHNAUER, T. A. (1959). Further studies on the proteolytic complex and the associated insect toxicity of *Bacillus larvae*. *Bact. Proc.* p. 21.

REINHART, J. F. (1942). *Nosema apis* in package bees. *Amer. Bee J.* **82**, 516 only.

RENNIE, J. (1923). Acarine disease explained. *Mem. N. Scot. agric. Coll.* no. 6.

RENNIE, J., WHITE, P. B. & HARVEY, E. J. (1921). Isle of Wight Disease in hive bees. *Proc. Roy. Soc. Edinb.* **52**, 737–779.

SARDAR SINGH (1956). Acarine disease in *Apis indica* F. *Indian J. Ent.* **18**, 458–459.

STURTEVANT, A. P. (1924). The development of American foul brood in relation to the metabolism of its causative organism. *J. Agric. Res.* **28**, 129–168.

STURTEVANT, A. P. (1932). Relation of commercial honey to the spread of American foul brood. *J. Agric. Res.* **45**, 257–285.

TARR, H. L. A. (1936a). *Bacillus alvei* and *Bacillus para-alvei*. *Zbl. Bakt.* **94**, 509–511.

TARR, H. L. A. (1936b). Studies on European foul brood of bees. II. The production of the disease experimentally. *Ann. appl. Biol.* **23**, 558–584.

TARR, H. L. A. (1937a). Studies on American foulbrood of bees. I. The relative pathogenicity of vegetative cells and endospores of *Bacillus larvae* for the brood of the bee. *Ann. appl. Biol.* **24**, 377–384.

TARR, H. L. A. (1937b). Studies on European foul brood of bees. III. Further experiments on the production of the disease. *Ann. appl. Biol.* **24**, 614–626.

TARR, H. L. A. (1938a). Studies on American foul brood of bees. II. The germination of the endospores of *Bacillus larvae* in media containing embryonic tissues. *Ann. appl. Biol.* **25**, 636–643.

https://doi.org/10.23637/ERADOC-1-92

TARR, H. L. A. (1938b). Studies on European foul brood of bees. IV. On the attempted cultivation of *Bacillus pluton*, the susceptibility of individual larvae to inoculation with this organism and its localisation within its host. *Ann. appl. Biol.* **25**, 815–821.
WHITE, G. F. (1907). The cause of American foulbrood. *Circ. U.S. Bur. Ent.* No. 94.
WHITE, G. F. (1912). The cause of European foul brood. *Circ. U.S. Bur. Ent.* No. 159.
WHITE, G. F. (1919). Nosema disease. *Bull. U.S. Dep. Agric.* No. 780.
WHITE, G. F. (1920). European foul brood. *Bull. U.S. Dep. Agric.* No. 810.
WOODROW, A. W. (1942). Susceptibility of honeybee larvae to individual inoculations with spores of *Bacillus larvae*. *J. econ. Ent.* **35**, 892–895.
WOODROW, A. W. & HOLST, E. C. (1942). The mechanism of colony resistance to American foul brood. *J. econ. Ent.* **35**, 327–330.

https://doi.org/10.23637/ERADOC-1-92