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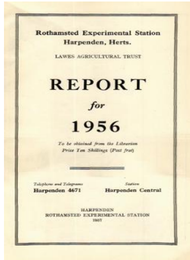
ROTHAMSTED RESEARCH

# Report for 1956






## Bees

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# BEE DEPARTMENT

C. G. BUTLER

## GENERAL

During 1956 the work of the department has largely followed the lines which have been outlined in previous reports. Several members of the department have lectured to Scientific Societies, Research Groups, Beekeepers' Associations, etc., and have taken part in Summer Schools and similar short courses; they have also continued to serve on various committees, such as the Ministry's Bee Disease Advisory Committee.

During the year J. Simpson was awarded the Ph.D. degree of London University, and C. R. Ribbands resigned in order to take up an appointment as Lecturer in Crop Pests in Cambridge.

## BEE BEHAVIOUR

*Swarming*

J. Simpson has continued his work on the factors controlling the incidence of swarming by honeybee colonies. Some of the results of the work have already been mentioned briefly in previous reports, and full accounts of these have now been prepared for publication.

Further work led to the conclusion that if colonies of bees were given adequate space in their hives, and were not interfered with in any way, the proportion which would swarm is probably between 10 and 40 per cent.

The numbers of colonies which swarm become smaller in the latter part of the summer in a way which cannot be related directly to seasonal changes in environmental conditions. Much of this reduction is probably due to exhaustion of the supply of colonies capable of swarming. An examination of the records of colonies which began to rear queens but did not complete the process provided little evidence that the tendency of colonies to rear queens diminished in the latter part of the summer in any way which could not be directly related to the seasonal reduction in length of day, temperature and availability of nectar. On the other hand, dissections of worker bees in 1954 showed that there was a seasonal trend in the size of their fat-bodies, which continued in the same direction from May until October, thus suggesting the existence of a physiological change which has no direct relationship with environmental conditions.

In this connection Simpson has also made comparative studies of the salivary glands of worker bees, both in and out of the swarming season. He has been attempting to determine the nature and function of the secretions of these glands, in particular of the post-cerebral and thoracic ones, about which little is known. He has found that the post-cerebral and the thoracic glands contain both oily and watery secretions, the two phases being clear, homogeneous and distinct. Previous workers have failed to find any invertase, diastase or protease in the secretions of either gland, which Simpson has shown in each case to be slightly acid (about pH 6). The oily contents of the secretions of these glands stain with Sudan III and readily dissolve in ether or chloroform, but not in alcohol or in acetic acid. They appear to be largely unsaponifiable. The quantity of oily material present in these secretions varied considerably from bee to bee. The glands of some contained no detectable amount, but most had at least a little; many bees had large amounts in their post-cerebral glands, but only a few had much in their thoracic glands.

In summer large amounts of this oily material were much more frequently found in the post-cerebral glands of bees caught whilst foraging than in those of bees taken from their hives.

Attempts are also being made to compare the properties of the saliva discharged by bees under various circumstances with those of the secretions of the various salivary glands. So far it appears that when saliva is used by bees to dissolve dry sugar this saliva is either neutral or only very slightly buffered and contains no invertase or reducing sugars. Such saliva might, therefore, come from either the post-cerebral or thoracic glands, but could not come from the mandibular glands, whose secretion is strongly acid, nor from the pharyngeal glands, whose secretion contains invertase, nor by regurgitation from the honey-stomach because its contents contain acids, invertase and reducing sugars. Filter-paper which bees had chewed was found to be distinctly alkaline (pH 8–9), although none of the salivary glands are known to produce alkaline secretions.

Simpson has observed that a strong scent, which several workers have found to be associated with the contents of the mandibular glands, occurs chiefly in the glands of foraging bees.

In view of the work of de Groot and Voogd (*Experientia*, 1954, **10** (9), 384), in which they demonstrated that queen substance is more abundant on the head of a queen than on other parts of her body, Simpson has suggested that this important substance may perhaps be secreted by the mandibular glands, which are much larger in the queen than in the worker. A preliminary experiment by C. G. Butler to test this hypothesis failed to yield a positive result, but further tests with an improved technique are being undertaken.

*The transmission of food between adult worker honeybees*

J. B. Free has obtained further information on the distribution of food throughout a colony by recording the passage of food between marked worker bees of known ages. He has found that day-old bees seldom feed other bees, and that two day-old bees do not give food as frequently as older bees. During the early part of adult life worker bees receive food more often than they give it, but as they grow older they tend to give it more frequently than they receive it.

As bees grow older the mean age of the bees who feed them increases; as does the mean age of the bees they themselves feed. In general, bees tend to receive food from bees older than themselves and to give it to bees younger than themselves. Thus there

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is a general tendency for food to pass from older bees, many of whom are foragers, to younger bees who feed the brood. However, bees of all ages are fed by, and feed, bees of a wide range of ages. It is most probable, therefore, that food, and any substance it may contain, such as social hormones, are passed freely around the colony between bees engaged in different duties.

Although bees which are about to feed other bees generally have more food in their honey-stomachs than those who are about to receive it, the quantities of food contained in the honey-stomachs of bees belonging to these two categories overlap considerably. Several factors may influence the result. For example, the food-offering behaviour of bees which have recently licked their queen, and so obtained queen substance, is not governed by the amount of food in their honey-stomachs. Bees undertaking certain duties may more readily retain food than those occupied with other duties. From a study of the behaviour patterns associated with food transmission it appears probable that those bees who require relatively large amounts of food in connection with wax or brood-food secretion can stimulate bees with little food to feed them, and, similarly, bees with full honey-stomachs (e.g., successful foragers) are sometimes able to stimulate bees which already contain a lot of food to accept more from them.

The quantities of food which different bees contain when they show themselves willing to give or receive food may well also depend on their past experience, since it was found that improvement or deterioration in the attractiveness, as well as in the quantity, of syrup fed to bees had a marked effect on the amount retained in their honey-stomachs. These results probably account for the fact that a much closer correlation between a bee's behaviour in respect to food transmission and the amount of food in her honey-stomach is obtained with recently emerged, unconditioned, bees than is obtained with bees of all ages taken at random from their colonies.

*Queen supersedure*

C. G. Butler has continued his studies on the process known as "queen supersedure", by means of which the worker bees of a colony replace their queen by a young one without swarming.

Many suggestions have been made in attempts to explain the initiation of this process, such as: disease of the queen; her inability to supply her colony with "sufficient" eggs either on account of old age or injury; the production of an abnormally high proportion of drones; failure of the queen to supply sufficient queen substance—and these have been investigated.

It has been possible to demonstrate that a queen can still inhibit worker bees from rearing further queens, even though she is not producing any eggs externally. Nevertheless, it was found that young virgin queens do not always inhibit queen rearing by the workers of their colonies, which cannot be explained by the fact that they are not producing any eggs. It was also found that queens which were laying drone-producing eggs only were often fully capable of inhibiting queen rearing; and it was demonstrated that a queen, who had actually been superseded by her workers, was nevertheless capable of inhibiting queen rearing in small, but not in large, colonies.

As it has been shown previously (Butler (1954), *Trans. R. ent. Soc. Lond.* **105**, 11) that a substance which worker bees obtain from their queens (queen substance) inhibits them from rearing further queens, provided that the quantity is sufficient for the number of bees present in their colonies, it is probable that this queen was producing insufficient queen substance to inhibit queen rearing in a large colony.

It was concluded that shortage of queen substance is the only immediate cause of queen supersedure, and that any other factors which tend to initiate this process do so by reducing the queen's output of this substance. A paper giving a full account of this work is being published.

*Queen substance*

Further work on the nature of queen substance and on its collection and distribution by the members of a colony has been carried out by C. G. Butler, who has shown that, despite suggestions to the contrary (Voogd, S. (1956) *Experientia*, **12**, 199), the queen substance which worker bees receive in regurgitated food from other workers, who have either obtained it directly by licking their queen or indirectly from other workers, is sufficient to inhibit development of their ovaries in the absence of a queen of any kind. However, it has become clear that a greater degree of ovary inhibition is obtained when an extract of queen substance is placed on the body of a dead worker bee, to which the experimental bees have free access, rather than in the food of the latter. This has resulted in an improvement in the technique of testing the efficacy of extracts from queens, etc., and various other substances, as inhibitors of ovary development in queenless worker honeybees.

In preliminary experiments it has been found that small quantities of both androsterone and testosterone (kindly supplied by Dr. R. K. Callow of the National Institute for Medical Research), when fed to worker honeybees, inhibit development of their ovaries under conditions in which they would otherwise develop. Materials extracted in acetone from the bodies of mated queen wasps (*Vespula germanica*) and mated queen ants (*Formica rufa* and *F. fusca*) have also been found to have similar inhibitory effects on ovary development to those obtained with honeybee queen substance when given to queenless worker honeybees; but extracts obtained from mated queen bumblebees failed to produce an inhibitory effect.

## General Research

*Ovary development in worker bumblebees*

J. B. Free has continued his study of egg-laying workers in bumblebee colonies. He has shown that the ovaries of workers kept singly or in small groups at low temperatures do not develop as much as those kept in the same way at higher temperatures. However, the degree of ovary development of the bees in the larger groups was similar at all the temperatures used. It seems possible, therefore, that in these larger groups the body temperatures of the individual bees are raised so that the retarding influence of cold on ovary development is no longer operative.

Nevertheless, the number of bees forming a group has an influence on the development of the ovaries of the members of the group when temperature ceases to be a limiting factor. Within limits the extent to which the ovaries of worker bumblebees develop increases with the size of the group. It is possible that this effect of group stimulation of ovary development is related to differences in the amount of food consumed by individuals belonging to groups of different sizes. However, even when worker bumblebees are kept in isolation their ovaries sometimes develop sufficiently for them to lay eggs.

*The defence of bumblebee colonies*

J. B. Free has studied the ability of bumblebees to recognize bees from other colonies as intruders. Bumblebees are able to recognize intruders by their scent alone in the same way as honeybees can. It seems possible that the distinctive odours of bumblebees from different colonies are partly genetical in origin and partly due to absorption of nest odours on the waxes of their body surfaces.

Guards are often present at the entrances to the nests of large colonies, but, although no guards are present in the case of a small colony, certain of its members are more ready to attack intruders than others. The degree of aggressiveness shown by individual bees appears to be related to the degree of development of their ovaries. This association between ovary development and ferocity also probably explains the hostility which queen bumblebees show towards each other. Free has suggested that the fact that the ovaries of all but one of the members of a bumblebee colony normally remain relatively undeveloped is of considerable importance in maintaining peace in the colony.

## Bee Diseases

*Nosema disease*

L. Bailey has continued his work on Nosema disease. In late April 1956 he found that there were only 11 colonies at Rothamsted, out of a total of 250, which were infected with this disease. One of these had 18 per cent of its bees infected, but the rest had less than 5 per cent infected. The original general infection has obviously dwindled, almost to extinction, as the result of fumigation of all combs with the vapour of acetic acid, thus killing all Nosema spores on them (see *Report Rothamst. exp. Sta. for* **1953**, **1954**, **1955**).

An experiment is now in progress to see if Nosema infection can be eliminated from colonies by fumigating all their combs, except those containing brood, in spring, and returning them to the colonies directly after fumigation is complete—i.e., after about one week. It is thought that the small number of brood combs remaining untreated may be cleaned sufficiently by the bees, who use them repeatedly during summer, to eliminate any residual infection. This method, if successful, will avoid the labour of transferring colonies completely to fumigated combs, and is likely to be more attractive to commercial honey farmers possessing large numbers of colonies.

*Acarine disease*

L. Bailey and Elizabeth Booth (*née* Carlisle) have, by marking in the autumn of 1955 all the bees of a colony infected with acarine disease and then taking periodical samples from them, shown that the percentage of infected individuals in this colony remained constant until about March, after which it declined slowly but significantly. This indicates that the infected bees were dying more quickly than the non-infected ones. In the case of a lightly infected colony this would be unimportant, but in the case of a heavily infected colony it would lead to a serious reduction in strength. This experiment is now being repeated with heavily infected colonies.

From mid-April onwards infection of the unmarked bees in the experimental colony mentioned above began to increase from 4 per cent at the constant arithmetical rate of about 20 per cent per month. This increase ceased shortly after the first period of intense activity of the colony, in May 1956, during a honey-flow; but it recommenced at the same rate when the honey-flow failed in June and continued until the end of August. Between June and September no honey-flow occurred, and infection of the bees reached 90 per cent. Although less-detailed observations were made on them, it became clear that infection followed a similar course in all the other colonies suffering from acarine disease.

In 1955, when the weather in summer was consistently good, a similar rate of increase of infection occurred from about April onwards, but it ceased and then continued to decrease after late May.

It seems, therefore, that suppression of infection is associated with high colony activity; hard-working, infected bees probably die off quickly, and there may be a partial separation of the older more infected bees from the newly emerged ones which have not yet become infected and which are known to be susceptible to infection only during the first few days of their lives.

*European Foul Brood*

The isolation in pure culture of *Streptococcus* ("*Bacillus*") *pluton* has now been accomplished by L. Bailey, who has published a short account of his work giving details of the medium employed (175). He has shown that both *S. pluton* and *Bacterium eurydice* have to be present together in a larva to cause European Foul Brood disease.

*S. pluton* is normally an anaerobe, but until recently *B. eurydice* was considered to be an aerobe or microaerophile. It is now evident, as a result of Bailey's work, however, that *B. eurydice* grows equally well, perhaps even better, as an anaerobe if fructose and glucose are both present in the medium : honey has the same effect. Anaerobic growth with any concentration of either fructose or glucose alone is feeble. The simultaneous growth of *S. pluton* and *B. eurydice* within the larval gut, in what are presumably anaerobic conditions, thus now becomes understandable.

The metabolic pathway of fructose would appear to be different from that of glucose in *B. eurydice*. The growth of *S. pluton* is the same with glucose or fructose or both these sugars together.

Bailey has obtained complete control of this disease by feeding quaternary ammonium compounds in sugar syrup to infected colonies in the autumn. Signs of reinfection returned as usual during the following season in control colonies, but no trace of this disease was found in the treated colonies, which were inspected at frequent intervals throughout the season. Dilutions as low as 1 part in 150,000 of di-*n*-decyldimethylammonium bromide fed in 3 gal. of syrup eliminated the disease from a colony which had suffered a most severe attack. The untreated control colony, which was derived from the same stock before treatment began, was almost annihilated by the disease.

These compounds are not toxic to bees until concentrations greater than 1 part in 15,000 are fed, and the effects on colonies which have overwintered with this concentration in their food stores has been exceedingly slight.

Large-scale trials are now in progress with this form of treatment, and results should become available towards the end of 1957.

In laboratory tests both *S. pluton* and *B. eurydice* have been found to be very sensitive to dilute solutions of quaternary ammonium compounds.

These compounds exert most of their effect in colonies by killing the dormant bacteria on the comb and in the stores during the winter. The disease is suppressed if these compounds are fed in syrup to infected colonies during the summer, but the effect is not permanent.