ROTHAMSTED RESEARCH

Report for 1959

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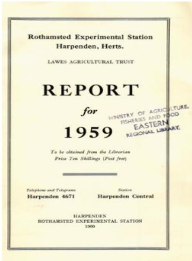

Bee Department

C. G. Butler

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BEE DEPARTMENT

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In September J. B. Free returned from Ontario Agricultural College, where he studied pollination problems for a year, and S. C. Jay, of the University of Manitoba, arrived to work on the nutritional and other requirements of the honeybee larva.

J. Simpson attended the foundation meeting of the European Committee of the Bee Research Association in Paris.

Members of the Department again lectured to scientific societies, beekeepers' associations, etc., and served on various committees.

Bee Behaviour

Swarming

The conclusion that swarm preparations are less frequent in colonies with queens reared in the current year than in those with queens of the previous year (Simpson, *J. agric. Sci.* **49** (1957), 387–393) was confirmed by analysing records kindly supplied by Mr. R. O. B. Manley, for these showed that colonies with 2-year-old queens prepared to swarm more than three times as often as did those with 1-year-old queens. (Simpson.)

Mated laying queens of colonies not rearing queens contain about four times as much queen substance as do either mated laying queens of swarms from uncrowded colonies or superseded queens, and queen rearing during swarming and supersedure preparations in uncrowded colonies probably results from insufficient queen substance. The amount of queen substance produced during a queen's laying life probably gradually decreases until it is too little to inhibit her colony from rearing queens. However, queens made to swarm by overcrowding colonies contain as much queen substance as do mated laying queens from colonies not rearing queens. Workers of colonies preparing to swarm have the same requirements for queen substance as have workers from colonies not preparing to swarm, so queen rearing in overcrowded colonies probably results from ineffective distribution of queen substance rather than from the queen producing too little. (Butler.)

In 1952 Simpson made a large colony swarm, when it only had one queen cell (containing an egg), by overcrowding it, and in 1959 he similarly made a small colony swarm without any accompanying queen rearing. Thus crowding can induce swarm emergence without queen rearing being an intermediate stage. Such swarming approaches the type called " absconding ", which is frequent with *Apis indica* but rare with *Apis mellifera*, in which *all* the bees leave their hive during unfavourable conditions without the colony reproducing.

Although queen rearing is always regulated by amount of queen substance, it is uncertain whether the shortage of queen substance leading to queen rearing in an uncrowded colony preparatory to swarming results from swarm preparations somehow changing the queen's production of queen substance, or whether the queen-substance deficiency initiates the swarm preparations. Experiments to discover whether an uncrowded colony that is rearing queens can be prevented from swarming by giving extra queen substance must wait until the substance is plentiful. An active fraction of queen substance was isolated in a crystalline form and partly identified (Butler, Callow and Johnston, *Nature* **184** (1959), p. 1871), and it is hoped that this will lead to its synthesis and tests of its applications in practical beekeeping.

Swarms were found to contain many bees that remembered the position of their parent colony and some that did not. When orientated bees were released a short distance from their swarm they returned to the site of their parent colony, demonstrating that swarming had neither obliterated their memory of the old site nor irreversibly changed their behaviour. Bees of a swarm hived a short distance from its parent colony do not return to the latter (unless their queen is taken away), but this is not because they have forgotten it. (Simpson.)

Queen balling

Worker bees sometimes surround a queen to form a " ball ". Such behaviour can result in the queen's death, but does not always do so, and it is often stated that there are two kinds of " balls ". We have now established this and found that in one kind the workers are aggressive and kill the queen, whereas in the other they " protect " her. Hostile balls were produced by removing queens from their colonies and returning them about 5 hours later or, in susceptible colonies, merely by introducing an alien odour, such as that of marked young bees or smoke. In these balls the workers tightly gripped the queen and other bees with their mandibles, vibrated their wings, produced high-pitched buzzing sounds and curved their abdomens round as though attempting to sting. The bees often lost their grip on the comb and the ball fell slowly to the hive floor, where the queen was killed. Benign balls occurred around virgin queens which had accidentally escaped from their cells. These balls were looser and did not fall from the comb. The workers forming such a ball did not buzz or vibrate their wings but licked at the queen herself and even at the place where she had touched the glass of the observation hive. The queen frequently escaped from these balls and appeared unhurt. The balls were similar to, but larger than, the clusters of bees which had previously prevented the queen from leaving their cells. (Simpson.)

Gland development and bee behaviour

About the time that worker bees begin foraging their hypopharyngeal glands retrogress, but redevelop should they need to feed brood. This was shown by capturing foragers at the hive entrance, confining them in groups in cages at 35° C. and giving them pollen-candy and water. When larvae were also present in a cage, and only then, the bees' hypopharyngeal glands redeveloped, showing that the protein (pollen) diet alone did not stimulate the glands but that larvae also needed to be present. (Free and Spencer-Booth.)

How diet during the first week of adult life affects the develop-

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ment of a worker's hypopharyngeal glands and its subsequent behaviour was studied. Newly emerged bees were divided into three groups. Two groups were caged in the hive, where one was fed on carbohydrate only, and the other on carbohydrate and protein (pollen); the third (control) group was left free in the hive. All the caged bees were released in the hive when 8 days old. The group fed on carbohydrate only began foraging earlier than the other two and, unlike the other two, the hypopharyngeal glands of this group never developed sufficiently for them to be likely to secrete brood food. Probably, therefore, these bees started foraging earlier because they omitted brood feeding from the normal sequence of duties. There was little difference in development or behaviour in the other two groups. (Free.)

Previous workers have found differences between the food given to young and old worker larvae. This could mean that the food given and the age of larvae fed changes as a nurse bee gets older. However, observations on the distribution of bees of known age on combs containing larvae of different ages showed that bees of each age were almost equally distributed on these combs irrespective of the age of the larvae. Therefore, it is likely that an individual bee varies the composition of the food to suit the age of the larva it is feeding. (Free.)

Drifting

Experiments to determine the way in which bees distinguish their own hives from those of other colonies were continued. Painting a square foot of each hive immediately above its entrance a distinguishing colour is about as effective in preventing drifting as is painting the entire hives different colours. Bees also learn something of the shapes of their hives because they drift more frequently to hives with the same number of brood chambers as their own than to hives of different shapes and sizes. (Free and Spencer-Booth.)

Temporary acceptance of larvae in queen cells by queenright colonies

At weekly intervals during the summer of 1957 ten young worker larvae, placed in artificial queen-cell cups in a frame as for queen rearing, were put between combs of young brood in apparently normal colonies without removing their mated laying queens or isolating them. Whenever a colony began to rear queens spontaneously it was discarded from the experiment. Thirteen colonies remained in the experiment continuously for 16 weeks; between 2 and 10 of them temporarily accepted 3–23% of the larvae in the queen cell cups in the various weeks. When the accepted larvae were left in the colonies, however, they were rejected after a few days. The number of larvae accepted varied significantly from week to week and colony to colony; one group of colonies, headed by queens bred from stock received from the U.S.S.R., was particularly inclined to accept larvae. No connexion could be shown between acceptance of larvae and weather, colony size or subsequent spontaneous rearing of queens. Larvae were accepted in February and April, and it is uncertain whether they are less readily accepted in winter than in summer. (Butler and Simpson.)

General Research

Mandibular glands of workers

The food of honeybee larvae contains a substantial amount of 10-hydroxy-Δ^2-decenoic acid. (Butenandt and Rembold, *Hoppe-Seyl. Z.* **308** (1957), 284.) Having studied the properties of the secretions of the various salivary glands of adult workers, Simpson (see publications) concluded that their mandibular glands were the most likely source of this substance. Glands were supplied to Dr. S. A. Barker and his colleagues at Birmingham University and to Dr. R. K. Callow and Miss N. C. Johnston of the National Institute for Medical Research, who previously isolated the principal component of mandibular gland secretion from an extract of worker bees' heads. Both groups independently confirmed the presence of 10-hydroxy-Δ^2-decenoic acid in the mandibular glands. Its occurrence as the main constituent of the secretion was shown by Dr. Callow and Miss Johnston, and its absence from the other salivary glands by Dr. Barker and his colleagues.

Male genitalia of Apis *species*

During attempts to obtain semen from *A. indica* drones to inseminate *A. mellifera* queens, important anatomical differences between the male genitalia of these two species were noted. These differences may prevent normal hybridisation. (Simpson.)

Queen substance production by virgin queens

Virgin queens sometimes fail to prevent even small colonies from rearing queens because they do not produce enough queen substance. This makes it difficult to replace the virgin queen of a colony by a mated laying queen, because when there is a low level of queen substance among workers it is difficult to make them accept a mated laying queen. (Butler.)

Wax extraction

Various methods of extracting beeswax from old brood combs were tested, but with the best tried at least 40% of the wax in the comb was lost in the dross. (Fairey.)

Pollination

Although insect pollination is probably necessary for a maximum crop of beans, substantial yields can be obtained without insects; a deficiency of pollination should be suspected when the pods are distributed throughout the length of the stems instead of being mostly at the bottom. (Riedel.)

Experiments of the type previously done with field beans and opium poppies to find the best time to take bees to the crop were extended to peach, sweet cherry, apple, bird's-foot trefoil, lucerne and red clover. The results were all similar and show that delaying taking bees to crops requiring pollination until they have begun to flower always increases the number of foragers, and is particularly beneficial with a crop that has a short flowering period or is less attractive to bees than other flowers in the district. (Free.)

Bee Diseases

European Foul Brood disease

Individually identified larvae that had their food inoculated, when they were less than a day old, with *Streptococcus pluton*, were mostly ejected before they were ready to be sealed in their cells by adult bees. Most ejected larvae were 4–5 days old, but a few became diseased when they were about 5 days old. There was no significant difference in these events when infection was with 100 or 1,000,000 bacterial cells. Many fewer larvae were ejected, and more diseased larvae appeared, when a large proportion of unsealed brood was removed from the colonies at the same time as they were infected, when the colonies were strengthened with adult bees from elsewhere or when the colonies had recently been queenless. Colonies given additional unsealed brood at the same time as they were infected removed more infected and uninfected larvae than usual, but ejected infected larvae preferentially. The cells of infected larvae that pupated successfully were sealed later; the resulting pupae weighed less than normal and voided many viable cells of *S. pluton* in their faeces. The factor underlying all these phenomena seems likely to be nutrition: abundance of larval food leads to pupation of more infected larvae, thus raising the number of cells of *S. pluton* in the colony; shortage of larval food leads to ejection of more infected larvae. Infected larvae that die are presumably in conditions between the two extremes. These events are no doubt connected with the acute annual epidemic of disease at the end of June in endemically infected colonies.

The few larvae less than 2 days old that pupated after infection left a faecal deposit rich in *S. pluton* in the form of lanceolate cocci in their cells. The larger number of survivors of larvae, infected with the same dose when 2 days old, left few bacteria, most of which appeared rod-like and reminiscent of *S. pluton* grown *in vitro* in the presence of oxygen.

Larvae were ejected as described above when inoculation was with *S. pluton* cultivated *in vitro*; *S. pluton* grown in the presence of 5% air was significantly less virulent (measured as the percentage of the infected larvae ejected) than when grown anaerobically. (Bailey.)

Diseased larvae contained many more *Bacterium eurydice* than did symptomless larvae from the same colony at the same time; but the incidence of *B. eurydice* did not diminish in healthy larvae until autumn. Although evidence still suggests that this organism facilitates the onset of visible disease in larvae infected with *Streptococcus pluton*, its incidence clearly does not influence natural diminution of European Foul Brood disease, which normally occurs in July. (Bailey and Lee.)

American Foul Brood disease

Individually identified larvae were infected when less than 1 day old with *Bacillus larvae*, as in the experiments described above with *Streptococcus pluton* and at the same time. Some unsealed infected larvae were ejected by the adult bees, but at approximately the same rate at all ages. The ratio of brood to adult bees seemed to have much less influence on the ejection of larvae infected with *B. larvae* than with *S. pluton*, and larvae that survived were sealed into their cells at the usual age and produced pupae of normal weight. Thus nutrition does not seem to be involved in infection with *B. larvae*: this is not unexpected because very few bacteria develop in infected unsealed larvae. Infected larvae probably become diseased from toxins produced by the few bacteria. The inoculated spores were obtained from the remains of larvae that had died of American Foul Brood disease. Such remains contain water-soluble toxins that could perhaps be a product of larval decomposition, but they did not cause ejection of the larvae in the experiment described, because identical results were produced by both washed and unwashed spores. (Bailey.)

Nosema disease

Infection with *Nosema apis* was more common than usual in England and Wales in the spring of 1959, and many beekeepers suggested that it was responsible for the abnormally heavy winter losses of colonies. However, no significant correlation was obtained when the degree of infection in surviving colonies in the various Rothamsted apiaries in spring was compared with the losses in these apiaries. Furthermore, there was no correlation between the diminution of colonies during the winter and the degree of infection in them in spring. Increased infection was expected after the bad season of 1958, and it undoubtedly contributed to losses, but it seems clear that it was not the general underlying cause. Other effects of a bad season, such as pollen shortage, may be suspected of being responsible.

The effect of increased environmental temperature on the natural incidence of infection in endemically infected colonies was examined. Healthy bees were marked, artificially infected and introduced in mid-summer to endemically infected colonies whose infection had been watched since early spring. After 2 weeks all surviving marked bees were infected and contained as many spores as did naturally infected bees at the height of the spring epidemic. Meanwhile natural infection in the colony continued to diminish as usual. Thus increased environmental temperature does not appear to play a significant part in the natural suppression of infection, as has been claimed elsewhere. (Bailey.)

Acarine disease

Events in 1959 confirmed previous conclusions: the winter mortality of colonies was significantly higher only in the few colonies with over 30 bees infested per cent; by the autumn, infection had fallen to the lowest level since systematic observations of untreated colonies began in 1951, which was expected after the outstandingly good season. Again, as with Nosema disease, there appears to be a positive connexion between heavy infestations and periods of queen-lessness or reduced brood rearing. (Bailey.)

Investigations began on the resistance of bees older than about 9 days to infestation with *Acarapis woodi*. Such resistance is widely attributed to increase in the stiffness of the hairs covering the entrance to the anterior pair of thoracic spiracles in older bees. Accordingly, old and young bees had the hairs shaved off one of their spiracles and were introduced into cages of heavily infested bees. The young bees became infested, with no difference between tracheae leading from shaved or unshaved spiracles, but old bees remained uninfested. Attempts to find the true cause of the resistance continue. (Lee.)