ROTHAMSTED RESEARCH

Report for 1960

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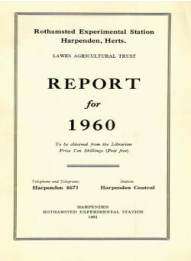

Bee Department

C. G. Butler

Bee Department, C. G. Butler (1961) Report For 1960, pp 168 - 175 **- DOI: https://doi.org/10.23637/ERADOC-1-93**

BEE DEPARTMENT

C. G. BUTLER

L. Bailey was awarded a Kellogg Fellowship, and spent four months visiting Universities and research centres in the U.S.A.

An exhibit on the pollination of fruit trees was shown at the Chelsea Flower Show, the Royal Agricultural Show, the Institute of Biology Annual Conversazione and the National Honey Show.

BEHAVIOUR AND PHYSIOLOGY

Swarming

Further evidence has been obtained to support the view that a queen, when grooming, distributes the queen substance secreted by her mandibular glands over her body surface, so supplying it to her workers and inhibiting them from queen rearing. In a series of experiments queens of colonies were prevented by collars from touching their mouthparts with any of their limbs. Although the mandibular glands of these queens contained as much queen substance as those of control queens, none was detectable on their bodies after a few days, whereas some was always detected on those without collars. (Butler.) The collars prevented the queens from moving around the combs and laying normally, and to overcome this other experiments were made in which the front legs of the queens of 13 colonies were amputated. The amputation led to the appearance of eggs in queen cells and to the rearing of queens. In August this was followed by the complete process of queen supersedure, in which the old queen survived until the young one was mated and laying. Earlier in the season the old (experimental) queens died naturally or were killed, before or soon after the young ones emerged from their cells and, although two of the colonies swarmed, they did so with young (virgin) queens. Whether removal of a queen's front legs interferes with queen substance distribution, or decreases the amount secreted is not yet known. (Simpson.)

The construction of a very large observation hive (16 British Standard brood combs in 6 tiers of 3) has made it possible to study the behaviour of colonies which swarm even though they are not crowded. Two colonies apparently preparing to swarm were put in this hive, and the queen in each died naturally, or was killed by the bees, before swarming occurred. One of the colonies did not swarm, the other did so with a virgin queen. The first worker bees to show excitement before the swarm emerged were in that part of the hive farthest from the entrance and at least 30 cm. from the queen or the nearest queen cell; thus the excitement was probably not caused by the queen, the queen cells or by bees who had inspected the weather outside. Nor was it caused by the queen departing on a mating flight, as about half the colony had left the hive before the queen reached the entrance tunnel, and she was

driven out by the workers while repeatedly trying to return. The workers did not actually push the queen, but those beside her stopped moving forward whenever she did, so that a mass of bees she could not penetrate, piled up behind her. Later, when mating flights occurred, the queen always ran straight through the entrance tunnel as soon as she found it, and the number of workers leaving the hive did not increase while she was going out. (Simpson.)

Old queens from 11 swarms from uncrowded hives each had only about a quarter as much queen substance as each of six mated laying queens from colonies free from queen rearing; in their content of queen substance they were indistinguishable from seven queens that had been superseded. (Butler.) Attempts are being made, therefore, to find difference in the behaviour of workers of uncrowded colonies in which queens are being reared preparatory to swarming and supersedure respectively. Observations will have to be continued for several years to seek consistent differences in behaviour. So far " piping " of queens, confinement of young adult queens in their cells by the workers, and dorso-ventral abdominal vibration dancing on queens, which are generally associated with swarming, have been seen in three supersedures when the old queen died naturally, or was killed by the workers, before or immediately after the young one emerged from her cell. These behaviour patterns have not been seen in complete supersedures when the old queen survived until the young one, who superseded her, had mated and begun laying. Thus incomplete supersedure may be intermediate between complete supersedure and swarming. (Simpson.)

Queen substance which, at the time of the last report, had been isolated and partly identified [Butler, Callow and Johnston, *Nature*, **184** (1959), p. 1871] has now been identified as 9-oxodec-2-enoic acid [$CH_3 \cdot CO \cdot (CH_2)_5 \cdot CH{:}CH \cdot COOH$] and synthesised [Callow, R. K. and Johnston, Norah C., *Bee World*, 1960, **41**, 152–153]. The synthetic material is as active in inhibiting ovary development in worker bees and queen rearing by them as the natural material isolated from the mandibular glands of queen honeybees. (Butler, Callow and Johnston.) However, the odour of 9-oxodec-2-enoic acid is not attractive to worker bees, whereas another substance that can be extracted from a queen in ethanol attracts workers a short distance away. Presumably, in the darkness of the hive, it helps workers near the queen to find her and so to obtain queen substance by licking her body. Work has begun, in collaboration with Dr. R. K. Callow and Miss N. C. Johnston, to isolate and identify the material responsible for a queen's attractive odour. (Butler.)

Ethanol extracts of *Apis indica* and *A. florea* queens (kindly supplied by Mr. L. A. S. Perera) inhibited queen rearing by *A. mellifera* workers, so queens of these species presumably also produce 9-oxodec-2-enoic acid in their mandibular glands. (Butler.)

Stinging

The factors provoking bees to sting were studied by comparing the number of times balls of cotton-wool, wrapped in muslin, were stung when treated in various ways and jerked over the top of an open hive or at a hive entrance.

Black balls were stung more than white against both black and

white backgrounds, and blue more than yellow against yellow or blue backgrounds. However, white balls were stung more than yellow against a yellow background and yellow balls were stung more than white against a white background, thus showing the effect of contrast.

Balls that had already been stung were more likely to be stung than fresh balls offered simultaneously, but the " sting odour " was effectively masked by " smoking " the balls. Bees stung balls that had been kept in their own hive, and so probably acquired something of their colony's odour, more than balls that had been kept in the hive of another colony. The reason for this is unknown. The scent of mammals and of human sweat provoked stinging, but treating balls with such insect repellents as dimethyl phthalate, citronellol and methyl salicylate decreased it.

Balls with half their surface areas covered with wool were stung more than muslin-covered balls, probably because of their texture. Also, the more rapidly a ball was moved, the greater the number of stings it received.

These results suggest that chances of being stung are decreased by wearing clean, smooth-textured, light-coloured clothing, by avoiding rapid movements and sweating, by using a repellent and by " smoking " any places that are stung to mask the " sting odour ". (Free.)

Queen rearing and introduction

The records of two honey-farmers (Messrs. R. O. B. Manley and A. S. Rowse) were analysed. Some of them show that queen cell cups containing newly grafted larvae were more readily accepted by queenless and broodless colonies than by queenless colonies with brood or by queenright colonies, and more readily accepted and completed by queenless colonies with brood than by queenright colonies. Acceptance was not influenced by the presence of unsealed queen cells in the recipient colonies or by the presence of food with the larvae in the cups.

Success in introducing sealed queen cells, virgin queens and mated laying queens to colonies was greatest when the queen of the recipient colony was either the same physiological age or a little younger. For example, sealed queen cells and virgin queens were more readily accepted by colonies that had been queenless for 3 days, and had probably started to produce queens of their own, than by colonies whose queens had been removed more recently. Again, cells from which queens were due to emerge within 4 days were more successfully introduced to colonies that had been queenless for several days than to colonies that had been queenless for shorter periods, and younger queen cells were more successfully introduced than older ones to recently dequeened colonies.

Mated laying queens were less successfully introduced to colonies from which virgin queens, queen cells, or a laying queen and queen cells had just been removed, than to colonies from which laying queens had just been removed.

The time of year was not correlated with the proportion of queen cell cups containing newly grafted larvae that were accepted and reared. During June and July the proportion of sealed queen

cells and virgin queens accepted was greater than at other times of year, but the proportion of mated laying queens accepted was smaller. The success achieved both in queen rearing and in the introduction of sealed queen cells and adult queens varied greatly from year to year. (Free and Spencer-Booth.)

The feeding of colonies

During two years colonies were fed either with dilute or concentrated sucrose syrup in spring, summer and autumn, and compared, during the week before feeding began, during the week of feeding and during the week after feeding, with unfed control colonies.

The amount of syrup taken increased as the season progressed. In each experiment more of the concentrated than of the dilute syrup was taken and, although the proportion of concentrated to dilute syrup accepted decreased between spring and autumn, the amount taken in spring was so great that the colonies receiving it obtained more water from it than those receiving dilute syrup. The concentration of the syrup made little difference to the proportion of sugar from it that was stored as " honey ".

The experimental colonies had significantly more brood both during and after feeding in 1958 with both syrup concentrations, but feeding had no detectable effect on brood rearing in 1959. The weather was more suitable for foraging in 1959 than in 1958; feeding syrup probably increases brood rearing when the weather is unfavourable for foraging, but has no effect when incoming food from natural sources is plentiful. The feeding of dilute or concentrated syrup probably lessened nectar-gathering when foraging conditions were good, and concentrated syrup may have lessened it more than dilute syrup.

While being fed, colonies collected increased amounts of pollen in both years. Pollen-gatherers are more valuable than nectar-gatherers in pollinating many crops (e.g., field beans, red clover, fruit trees), so the practice of feeding syrup to colonies that are taken to such crops will probably increase the amount of pollen they collect, and hence their efficiency as pollinators. (Free and Spencer-Booth.)

Bees can ingest dry sugar by dissolving it, or reducing it to a paste, with saliva from their labial glands (not with material from their honey-stomachs). The bees of a colony in an observation hive took granulated sugar slowly from a feeder of the Miller type. At first few bees licked the sugar, but later the whole surface became covered with feeding bees. Many bees, however, picked up grains of sugar in their mandibles, carried them to the hive entrance and flew away with them, presumably dropping them eventually. The proportion of sugar lost in this way has not yet been determined, but may be enough to make this method of feeding uneconomical. Icing sugar was not carried out by the bees, but proved unsuitable, as the bees became so heavily dusted with it that they could not go on feeding. (Simpson.)

Effect of cold

Experiments were made to find what factors determine the highest temperatures at which worker honeybees are immobilised by cold (chill coma temperature) and killed by cold (cold death temperature). The chill coma temperature for workers varied between +8° and +11° and was in general lower than that for queens and drones. Workers kept for some time at 20° had lower chill coma temperatures and recovered more rapidly from chill coma than workers that had been kept at 35°. Conditioning to a given temperature was complete after 24 hours.

Bees taken directly from the hive had a lower chill coma temperature in winter than in summer, but after conditioning at a given temperature for 24 hours this difference disappeared, so winter bees seem only temporarily conditioned to lower temperatures and not to have an intrinsically low chill coma temperature. The chill coma temperature of a bee decreased with age, which could partly be explained by the fact that older bees tend to keep away from the brood area (where the temperature is highest) when in the hive and so become conditioned to lower temperatures than younger bees who frequent the brood area. Nevertheless, even after conditioning at 35° for 24 hours old bees still had lower chill coma temperatures than younger ones, although the difference was smaller.

Most workers died after 50 hours in chill coma, and few lived more than 80 hours. More survived at +5° than at 0° or +10°. Cold death occurred between −2° and −6°. Between −3° and −5° the death rate increased as duration of exposure was increased from 30 to 180 minutes. The temperature to which the bees had become conditioned apparently did not affect the cold death temperature.

Apis indica workers entered chill coma at temperatures slightly higher than *Apis mellifera* workers. Groups of both *A. indica* and *A. mellifera* workers responded to low temperatures by eating more food, indicating increased metabolism and heat production. (Free and Spencer-Booth.)

Functions of salivary glands of adults

Invertase is secreted by the hypopharyngeal glands of worker honeybees, but not by their labial or mandibular glands. Queens and drones have no hypopharyngeal glands, and it has now been confirmed that their labial and mandibular glands contain no invertase, so they evidently have no salivary invertase. All honeybees secrete invertase in their mid-guts, and this enzyme is presumably necessary in the saliva of workers to invert sucrose during the ripening of nectar, which does not enter the mid-gut. (Simpson and Riedel.)

The mandibular glands of workers contain much 10-hydroxy-Δ^2-decenoic acid, which is abundant in the food they give to larvae. Wax and stored pollen were tested for the presence of strongly acid substances, but none were found, so worker bees are unlikely to use the secretion of their mandibular glands when working wax or storing pollen.

The mandibular gland contents of older bees have a strong smell,

and repelled foraging bees when added experimentally to a dish containing food. (Simpson.)

Pollination

Studies of the behaviour of honeybees visiting apple, apricot, peach, pear, plum and sweet cherry flowers showed that, when seeking pollen only from a flower, a bee scrabbled over the anthers, pulling at them with legs and mandibles. When collecting nectar a bee stood either on the stamens or petals and pushed its tongue and head towards the nectary. About 50% of the bees seeking nectar collected pollen as well, apparently incidentally, when it was abundant. On any one trip a bee usually collected either pollen only, nectar only or nectar with some pollen incidentally. The proportions of foragers behaving in these three different ways varied, not only from day to day, but also at different times on the same day. Except on pear trees, bees that were collecting pollen only visited more flowers per minute than those that were collecting nectar.

Bees that scrabbled for pollen, and nectar-gatherers that approached the nectaries while standing on the stamens, touched both anthers and stigma and were probably efficient pollinators, but nectar-gatherers that stood on the petals and approached the nectaries from the side were inefficient, as they touched only the anthers of the apricot, pear, cherry, plum and pear flowers, which had spreading filaments, and did not even touch these in the apple flowers which had upright filaments. In apple varieties with relatively short and thin filaments most of the nectar-gatherers preferred to push their way to the nectaries from the top of the flower, but in varieties with thicker and longer filaments they preferred to approach the nectary from the side. The filaments of Bramley Seedling were so tall and thick that would-be nectar-gatherers could not reach the nectaries either from the top or the side of the flower. In this variety, therefore, it is unlikely that nectar-gatherers are of much use as pollinators, but, of course, pollen-gatherers make excellent pollinators, both in this and other varieties.

On average a bee visited slightly under two trees per trip and tended to move to a tree immediately adjacent to that on which it started working. It is possible, therefore, that when more than two rows of trees of the main variety are planted between rows of a polliniser variety the centre row, or rows, sometimes get pollinated less than those adjacent to the polliniser. In orchards where adjacent rows of trees were farther apart than adjacent trees in a row the bees tended to move more readily between trees in the same row than between trees in adjacent rows. (Free.)

Bee Diseases

European Foul Brood disease

As *Streptococcus pluton* survives long periods (now known to be at least 3 years) in dried smears of infected larval mid-guts, it was possible to study further methods of isolating it *in vitro* from natural material, and to observe its behaviour, which changes somewhat in subculture. Isolation was often better in deep agar (1%

Difco yeast; 1% glucose; 1·36% KH_2PO_4; 2% agar; pH to 6·6 with KOH and sterilised at 116° for 20 minutes) incubated at 35° in a normal atmosphere, than on plates of the same agar incubated anaerobically with CO_2. It was consistently better in deep agar sterilised without glucose than with. In medium made by mixing separately autoclaved agar and 20% glucose solution containing 0·01% phosphoric acid, growth was often in two bands: one reached from, or near, the bottom of the tube to a sharply defined limit about a centimetre from the surface, and this was followed by a narrow band above it and separated from it by a blank interval a few millimetres wide. The number of cells in the inoculum did not greatly influence these phenomena, which also appeared earlier, but in the same form, when CO_2 was added to the atmosphere. Double-banded growth also arose from inocula composed of single colonies from pure subcultures. Heat-degradation products of glucose seem to stimulate growth, as Seitz-filtered glucose solutions gave uncertain growth which also had poorly demarcated limits and was not in separate bands.

Isolation tests in plates, anaerobically $+CO_2$, have recently given erratic results, and growth has often failed completely, whereas simultaneous tests with deep agar, as described above, gave satisfactory growth. The reason for these differences remains obscure, but is suspected to be variable amounts of inhibitory substances produced in the media; incorporating soluble starch (1%) invariably gave excellent isolation, even from very dilute inocula, presumably by neutralising the inhibitors. Isolation in deep agar was also greatly improved by adding starch. Subcultures seemed much less sensitive to the inhibitors. With starch, a rare strain of *S. pluton* was isolated and propagated on media containing little phosphate (0·01*M*).

Strains of *S. pluton* from diseased larvae collected in Louisiana and New York States, U.S.A., responded to the media described above in the same way as *S. pluton* collected in the United Kingdom, but they are more sensitive to the suspected inhibitors.

Isolation and propagation of Swiss strains of *S. pluton* at Liebefeld, Switzerland, have been found to be facilitated by using the original Rothamsted medium with added trace elements (a supplemented Hoagland's solution), but isolation and growth of all strains of *S. pluton* tested at Rothamsted, on media with or without starch, were not influenced by this modification.

After finding that European Foul Brood (EFB) disease and larval nutrition are closely involved, newly formed healthy pupae were weighed throughout the season to find out what variations occurred. Significant fluctuations were observed, with the same trends, simultaneously in different colonies, indicating an environmental effect. Weight decreased greatly in May, after a maximum at the end of April, coinciding with intense foraging activities and the first major outbreaks of EFB in Britain in the season. This is compatible with experimental results suggesting that acute outbreaks of EFB coincide with rapid increases of foraging and brood rearing activity, particularly after some brood restriction, when larvae may receive less glandular food than usual from the workers. Pupal weights were lowest at the end of July, and increased during

late summer and autumn, even though colonies were by then reluctant to rear much brood, as shown by the disappearance of many eggs and young larvae whose positions had been recorded. (Bailey.)

Nosema disease

Laboratory experiments were made to test claims that *Nosema apis* infection can be successfully treated by feeding colonies with common salt in syrup. Sodium chloride, in various concentrations in syrup, was fed to caged adult bees and 0·1% (w/v)—i.e., $\frac{1}{40}$ the concentration alleged to have been effective—was highly toxic to the bees. Controlled tests with salt in concentration of 0·1% and lower, non-toxic concentrations, on artificially infected caged bees, had no effect on the number of spores that developed in the bees' mid-guts. Toxic materials may, of course, kill mostly the older heavily infected bees and so lower the percentage of bees infected, but even if this is so, it seemed unlikely to be of net benefit to a colony.

Similar tests were made with the following materials, some of which have been reported by various investigators to have had beneficial effects in field trials (trade or abbreviated names, followed by manufacturers' names in parenthesis are used): Pentamidine isethionate, Propamidine, Embazin (May & Baker); Paludrine, Mepacrine (Imperial Chemical Industries); Daraprim (Wellcome Laboratories); Camoquin (Parke Davis & Co.); Griseofulvin (Glaxo Laboratories). All proved ineffective against *Nosema apis*. Merthiolate (Eli Lilley & Co.) and Nosemack (Heinrich Mack) had identical effects: at concentrations higher than 2 mg.% they suppressed infection, but they also shortened the lives of the bees significantly. (Bailey.)