ROTHAMSTED RESEARCH

Rothamsted Report for 1967

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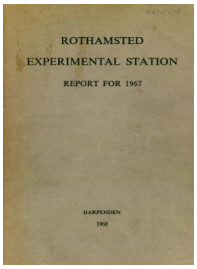

Bee Department

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BEE DEPARTMENT

C. G. BUTLER

Behaviour and physiology

Swarming. Colonies usually swarm when their worker bees prevent young queens from emerging from their cells and the queens make the sound known as "piping". Earlier evidence (*Rothamsted Report* for 1964, p. 195) that the piping sound, or at least something emanating from piping queens, tends to make colonies swarm was supported by the fact that when queens confined in cages were put into colonies only some piped and only these colonies swarmed.

Last year's attempt to investigate the factors that determine whether or not a colony confines its young queens in their cells (*Rothamsted Report* for 1966, pp. 208–209) was repeated with improved methods. It proved difficult to test separately the effects of colony size, of adult bee congestion and of abundance of young bees, but one or more of these factors did induce queen confinement. Depriving a colony of its flying bees a week before its queen cells were due to hatch increased the likelihood that the bees would destroy the remaining queen cells when the first queen emerged and so make confinement impossible. This supports the common recommendation to beekeepers to remove a colony's flying bees to prevent second and later swarms emerging.

Queen supersedure. Attempts were made to discover the conditions under which virgin and laying queens sometimes live together in colonies. In August and September small colonies were dequeened and, at various intervals after the first queen cells they produced had been sealed, their queens were returned in wire-gauze cages from which, after various further periods, their workers were allowed to release them by chewing through newspaper with which the mouths of the cages were covered. The returned queens did not induce workers to destroy queen cells while they were still caged, and only one queen was killed when released. When the release was early the colony quickly destroyed its queen cells, but when it was delayed until shortly before or after the first queen cell hatched sometimes the old queen survived and sometimes the young one. The two never lived together for more than 24 hours. (Simpson and Cherry)

Pheromones of queen honeybees. It has been known for several years that when a swarm of honeybees is in flight the worker bees are attracted to the queen by the odour of material produced in her head, and so possibly in her mandibular glands. However, experiments using techniques whereby the attractiveness to worker bees of the odours of several substances had been demonstrated, failed to show that the odours of any substances known to occur in the secretion of these glands, including 9-oxodecenoic and 9-hydroxydecenoic acids, were attractive to queenless workers.

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A chance observation, that workers of a flying swarm were interested in a piece of string lightly contaminated with 9-oxodecenoic acid and attached to a pole about 5 ft above the ground, led to further investigations being made with flying swarms in the field. These showed that, although flying workers were attracted by the odour of 9-oxodecenoic acid placed in a small wire-gauze cage attached to a pole 4 ft above the ground, they seldom alighted, or clustered, on the cage. The odour of 9-hydroxydecenoic acid was also attractive, but less so than the odour of 9-oxodecenoic acid. However, the odour of a mixture of these two acids was at least as attractive as the odour of live mated (or virgin) queens, and large, quiet clusters quickly formed on cages containing them, so we conclude that it is the combined odours of these acids that enable worker bees to follow their queen when swarming. In contrast, further tests have emphasised that it is not these odours, either separately or together, that attract workers in the hive towards the queen. (Butler and Simpson)

The power of 9-oxodecenoic acid to inhibit queen rearing and development of workers' ovaries is much increased by one or more adjuvant substances produced both by mated, laying queens and, although to a much smaller extent, by virgin queens. Efforts to identify this "inhibitory scent", which together with a queen's 9-oxodecenoic acid constitutes her "queen substance", have hitherto failed. However, the discovery that the scent of the queen that enables swarming workers to find her consists of the odour of a mixture of 9-oxodecenoic and 9-hydroxydecenoic acids suggested that the odour of 9-hydroxydecenoic acid might constitute "inhibitory scent". Laboratory and apiary tests have confirmed this. (Butler and Callow, Insecticides Department)

It is interesting that several distinct behaviour patterns in bees are induced by 9-oxodecenoic acid, the pattern elicited depending on the circumstances in which the acid is presented to them. In the hive it plays a major part in inhibiting queen rearing and the development of workers' ovaries, whereas in the field it helps swarming workers to find their queen and, when presented by virgin queens at greater altitudes (between 10 and several hundred feet), attracts drones but not workers, and also acts as an aphrodisiac when a drone finds a queen. Similarly, the odour of 9-hydroxydecenoic acid not only has the functions already mentioned but also stabilises a cluster of bees and helps maintain their social cohesion.

It seems that these pheromones might be useful in improving methods of pollinating fruit and seed crops. Small packages of adult bees in cardboard or plastic boxes, without a queen or brood but provided with a supply of pheromones, could readily be distributed through the crop to be pollinated and afterwards destroyed. The use of such standard pollinating units should not only be cheaper and easier than carrying hives into and out of the crops but would lessen the risk of the colonies contracting diseases or being damaged by pesticides applied to the crop. (Butler)

In parts of Austria, Germany and some other countries flying drones aggregate in the same restricted areas year after year, but attempts to find similar aggregation places in various parts of England, including fenland, woodland, arable and grassland areas, have failed. At the places surveyed regularly during the summer of 1967 the number of drones visiting on

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consecutive days when drones were flying very strongly ranged from a dozen or so to many thousands. (Butler and Watler)

Several years ago evidence was obtained that individual honeybee queens of the same age differ considerably in their power to inhibit queen rearing. The only techniques then developed to measure the inhibitory power of a queen either took a long time or involved killing her, and so could not be used to select breeder queens. Such selection may now become possible for a new method of measuring this power of a queen, which gives results overnight, has been developed, and its reliability is now being tested. (Butler)

Pheromones of worker honeybees. A pheromone by which worker honeybees recognise the entrances of their hives was demonstrated, and attempts are being made to isolate and identify it. A similar but distinct pheromone that marks nest entrances was also found in wasps. (Butler, Calam, Insecticides Department, Watler with Dr. D. J. C. Fletcher, University of Natal)

Brood rearing and food consumption during winter. One hundred and forty-nine colonies of honeybees, from a total of 13 apiaries, near Harpenden, were examined in October and again at the end of the following April to estimate the number of adult bees and the amount of brood present. On both occasions the hives of bees were weighed, and the weight of honey consumed by a colony during the winter was assumed to be the difference between the autumn and spring weights.

The amount of honey consumed per bee decreased with increase in the size of its colony, especially for colonies up to 18000 bees strong; thereafter any further decrease was relatively slight. Therefore, where large numbers of bees are required early in the season it is more economical to overwinter large than small colonies. Presumably bees in small colonies consume more food because they have a relatively greater cluster surface from which to lose heat. The five colonies that died during the winter were those with fewest adult bees in autumn. Excluding these five, the percentage of the autumn population that survived until the spring was similar for colonies of different sizes. However, the ratio brood/bees was greater for small than for large colonies both in autumn and spring. Probably this increased brood rearing in small colonies compensated for any shortening of the life of adult bees because of their greater metabolism during winter. Colonies headed by old queens stopped rearing brood earlier in the autumn than colonies headed by young queens. Probably the amount of brood reared in a colony in late autumn or early spring is associated with the amount of pheromones the individual bees receive from their queen. (Free and Racey)

Pollination and field behaviour

Effect of weather on foraging. This is being investigated with an apparatus that continuously records the level of sugar syrup in a feeder from which only one bee at a time can feed; the method allows the influence of weather

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on bee activity to be studied independently of its influence on the productivity of forage plants. At temperatures above about 13° C bees foraged continuously from sunrise to sunset. They sometimes began foraging at sunrise when it was colder (down to 3° C), but usually they did not. Apparently bees will not begin to forage at low temperatures unless other factors are favourable. (Simpson and Cherry)

Foraging behaviour on dandelions. Dandelion pollen formed a large proportion of the pollen trapped from colonies in orchards, particularly on cool days. A few bees visiting dandelion actively sought pollen, but most of those with pollen loads were nectar-gatherers that had collected pollen incidentally. Some of these nectar-gatherers packed the pollen into their pollen-baskets, but others discarded it, often when hovering. The proportion of foragers with pollen was greater in the morning, when it is more abundant, than in the afternoon. Some bees continued to visit dandelion for nectar only, long after it had finished yielding pollen for the day.

Many bees visiting dandelion and fruit flowers in orchards were given distinguishing paint marks. Most remained constant to the kind of flower on which they were marked, especially on any one day. Even on warm days when the dandelions closed soon after midday, very few of the pollen-gatherers that had been visiting them moved to fruit flowers, though the total income of fruit pollen sometimes increased in the afternoon. The dandelions, even when they closed early, clearly prevented many bees from visiting fruit flowers. Further, *Andrena haemorrhoa* the most abundant species of solitary bee visiting the dandelions, often collects pollen from fruit trees, so dandelion also competes with fruit trees for the visits of this bee. It is clear, therefore, that pollination of fruit trees is likely to be increased by destroying dandelions growing in or near orchards.

Strawberry pollination. The effect of honeybees on the pollination of strawberries (var. Favourite) was studied by comparing the weight and number of berries produced from: (*a*) plots enclosed in cages, each with a small honeybee colony; (*b*) plots in cages without bees; (*c*) uncaged plots in the open. There were no differences between the yields of berries from the plots caged with bees and from uncaged ones. The plots caged without bees set slightly fewer and smaller berries than the plots bees visited, but the greatest difference was that those without bees produced two or three times as many malformed berries because of inadequate pollination. Therefore, although this variety of strawberry can be partially pollinated without insects, probably by pollen falling from the anthers on to the stigmas, insect visits are necessary to produce the best results.

Foraging behaviour of bees on blackcurrant, raspberry and strawberry. There were more bumblebees than honeybees on blackcurrants, especially in cool weather, when sometimes there were no honeybees. Also, although the number of bumblebees fluctuated with changes of temperature during the day, they did so less than honeybees, whose numbers on days when they did work the flowers increased and decreased rapidly. Raspberry was the most attractive crop to bees, and was visited by many more honeybees than

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bumblebees. Very few bumblebees visited strawberry, and cold deterred honeybees from doing so.

The amount of strawberry pollen collected, and its percentage of the total collected, increased as the temperature rose and foraging conditions became more favourable. Many bees scrabbled for strawberry pollen and did not collect nectar. The percentage of raspberry pollen collected by colonies was greater at the beginning and end of a day's foraging than at an intermediate time when the most bees were visiting the flowers. Honeybees that collected nectar from raspberry collected pollen only incidentally; as happens when bees visit sunflowers and dandelions, some retained the pollen that collected on their bodies, whereas others discarded it, and individuals tended to be constant to one or other type of behaviour.

Few bumblebees and very few honeybees collected pollen from blackcurrant. Even honeybee colonies sited in large plantations collected little blackcurrant pollen, though slightly more than colonies farther away. Either bees discard blackcurrant pollen or it is so scanty that visible amounts seldom accumulate on their bodies.

Nearly all the bees that visited blackcurrant, strawberry and raspberry flowers touched both their stamens and stigmas, and so could have pollinated them. A bee visited only a small proportion of the open flowers of a blackcurrant bush or strawberry plant before moving on to the next. This habit makes them good cross-pollinators. A bumblebee working blackcurrant visited more flowers per cluster and more flowers per bush than a honeybee, but because it visited more flowers per trip, the size of its foraging area per trip was about the same as that of a honeybee.

On each of these three crops a bumblebee or honeybee tended to keep to plants in the same row throughout a trip, so when varieties that benefit from cross-pollination are grown they should be planted to allow cross-pollination within rows. (Free)

Bee diseases and pests

Paralysis. Several apparently normal queens were reared from larvae taken from colonies severely affected by paralysis. About 50% of dead bees collected in October from colonies headed since July by these queens contained much chronic bee-paralysis virus (CBPV), compared with less than 25% of dead bees from colonies headed by ordinary queens. Queens reared from paralysis colonies did not produce significantly more bees killed by all causes than ordinary queens, however, and neither type of queen produced more bees killed by CBPV than usual after they were fed the virus. Also queens reared from paralysis colonies and fed much CBPV when larvae did not produce unusually many paralytic workers.

Out of 11 further queens reared from larvae taken from a colony with severe paralysis, one became paralytic about a week after producing normal brood. A serological test showed she contained as much CBPV as a worker paralysed in nature or killed by injected CBPV. The single queen reared from her brood, however, remained normal and produced apparently normal workers.

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A convenient serological method of diagnosing paralysis in individual bees was devised. Antiserum prepared in rabbits against CBPV was absorbed with 7 volumes of the clarified extract of healthy bees (10 bees/ml saline + ¼ vol ether + ¼ vol CCl_4) and used in Micro-Ouchterlony gel-diffusion plates against the crude extract of the head of a bee ground in 1 drop of ether + 0·05 ml saline. A dense single line of precipitate formed in the agar with naturally paralysed bees or bees killed with injected CBPV. Antiserum prepared against acute bee-paralysis virus (ABPV) gave equally good results with bees killed by ABPV. Precipitin lines from extracts of heads of bees killed by CBPV and ABPV diffused across each other in the same gel-diffusion plate, confirming previous evidence that the two viruses are serologically unrelated.

Using serology to measure the virus content of infected bees, the effect was studied of changing temperature on the multiplication of the two viruses in bees infected with one or other or both. At 35° C ABPV on its own reached a larger concentration than at 30° C, but killed bees more slowly; its multiplication was unaffected at 30° C by simultaneous infection with CBPV, but was lowered at 35° C. In contrast, CBPV multiplied as much at 35° C with ABPV as on its own, but multiplied less at 30° C in doubly infected bees; CBPV killed bees sooner at 35° C than at 30° C though it reached larger amounts at 30° C. (Bailey)

Extracts of brains of acutely and chronically paralysed bees contained much infective ABPV and CBPV, respectively, and particles resembling those of ABPV were seen by electron microscopy in sections of the mushroom bodies of acutely paralysed bees. Larger crystalline masses of these particles were seen in midbrains, but they were less frequent and unevenly distributed. Similar particles were not seen in material from healthy or chronically paralysed bees. Particles somewhat resembling those of CBPV were seen with about equal frequency in brain tissue from healthy and acutely or chronically paralysed bees, but these particles also resembled synaptic vesicles, which are normal cell organelles, so that the distribution of CBPV particles in the brains of diseased bees remains in doubt. (Bailey and Milne, Plant Pathology Department)

Several attempts to isolate ABPV from bees from Australia failed, both with live bees sent from there to Rothamsted and with extracts of bees that had been injected in Australia with concentrated extracts of apparently healthy bees, a method that causes ABPV to multiply in British bees. (Bailey and Gibbs, Plant Pathology Department)

Sacbrood. Severe sacbrood seems linked with some queens, which may, therefore, transmit sacbrood virus (SBV), but four queens injected with SBV continued to produce normal offspring. Many attempts to show that SBV multiplies in adult worker bees also failed. Extracts of either the heads of adult bees or of their faeces were not infective to larvae a few days after the adults had eaten very many particles of SBV. Inocula from adult bees serially injected with SBV usually remained infective till the calculated effect of dilution by successive passage had brought their virus content to about 10^2 particles. Sooner or later, ABPV multiplied in bees serially injected with SBV.

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European foulbrood. Workers elsewhere have observed isometric virus-like particles in extracts of larvae alleged to have European foulbrood (EFB) and have said the disease is possibly caused by a virus. Bacteria-free extracts of samples of larvae from colonies in three separate parts of Britain and diagnosed as suffering from EFB by the National Agricultural Advisory Service contained no virus-like particles and were not infective in tests with individual larvae in normal colonies. All samples contained very many cells of *Streptococcus pluton*. It seems likely that the virus-like particles seen in extracts of larva with EFB were of sacbrood virus, which they resemble in shape and size and are common in apparently healthy colonies. (Bailey and Woods, Plant Pathology Department)

Streptococcus pluton was isolated in Denmark from colonies with EFB, and pure cultures sprayed into healthy colonies caused EFB. The inoculum and cultures of *S. pluton* reisolated from the artificially infected colonies were very closely related serologically to strains of *S. pluton* isolated in Britain. (Bailey with Dr. Niels Locher, Statens Biavlsforsøg, Denmark)

S. pluton was also isolated from larvae diagnosed as suffering from EFB in Brazil and the U.S.A. (Colorado and Massachusetts), and sent to Rothamsted. All strains were closely related serologically to strains isolated in Britain. The sensitivity *in vitro* of the U.S.A. strains to oxytetracycline, which had been used for many years among the colonies concerned for the treatment of EFB, was the same as that of local strains that had not been exposed to the antibiotic. (Bailey)

***Nosema apis* and *Malpighamoeba mellificae*.** A survey of colonies in England infected by *M. mellificae* showed that most bees in them were also infected with *N. apis*; bees in colonies with every individual infected with *N. apis* contained more spores of this parasite the more were infected with *M. mellificae*, and more apparently normal colonies than could be accounted for by chance were infected with both parasites. The two parasites are independent, however, because a few become severely infected with *M. mellificae* only, and many become severely infected with *N. apis* only. Colonies most infected with *N. apis* are those likely to have suffered most from "dysentery" (*Rothamsted Report* for 1964, p. 201) and this also spreads *M. mellificae* cysts. The average number of *M. mellificae* cysts per infected bee was, however, only $\frac{1}{50}$ the average number of spores of *N. apis* per infected bee, so *M. mellificae* probably spreads only when dysentery is more severe than is needed to spread infection by *N. apis*. Many individual bees infected with both parasites seemed normal. Foraging bees were among those containing most spores and cysts per individual. Sickness of colonies infected with *M. mellificae* may therefore be caused primarily by factors other than infection but also associated with dysentery, as it seems to be when colonies are severely infected with *N. apis*.

The regression of the percentage (transformed to probits) of bees infected with either *N. apis* or *M. mellificae* on the logarithm of the number of spores or cysts in samples of 25 bees was highly significant. The regression was about the same for samples taken of dead and live bees at different times of the year. To count the spores in samples seems a quick

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and useful compromise, therefore, between examining samples merely for the presence or absence of infection and the very laborious method of examining individual bees to estimate the percentage of a population infected. (Bailey)

Pathology of other insects

Wheat-bulb fly. Gravid female wheat-bulb flies, captured in the field, laid 28·2 eggs/fly at a rate of 1·6 eggs/fly/day, when, in addition to their basic food of diluted honey and condensed milk, they were fed a spore suspension of *Septomyxa affinis* (see Jones, Entomology Department, p. 202) and 20·5 eggs/fly at 1·2 eggs/fly/day when fed a spore suspension of a species of *Phialophora* which sometimes infects wheat-bulb fly eggs. Flies fed the more usual citrated beef blood yielded 23·0 eggs/fly at 1·7 eggs/fly/day. Flies mated in the laboratory or in emergence cages in the field laid slightly fewer eggs when fed a growing culture of *S. affinis* than when fed blood, but many females, especially those fed *S. affinis*, succumbed to pathogenic fungi. This supports the view that fungal spores, mainly of *S. affinis*, which fill the crops of many wheat-bulb flies caught in the field, are a major source of food for them.

More than 1000 gravid female wheat-bulb flies were obtained in eight samples taken in Hertfordshire between 26 July and 17 August 1967. These flies were kept in an outside insectary, supplied with suitable food and examined for species of the fungus genus *Entomophthora* when they died.

An average of 2·4% (range 0·0–11·5%) of the flies died within 5 days of capture and were infected with *Entomophthora muscae* Cohn. One fly was infected with another species of *Entomophthora*, probably *E. dipterigena* Thaxter. These flies may have infected others caged with them, of which 0·8% were infected with *E. muscae* and 0·2% with the *E. dipterigena*-like species. The abdomens of 0·9% of flies were very distended ventrally, their contents consisting almost entirely of spherical entomophthorous resting spores about 37·5 μ in diameter; the abdomens of 0·7% that seemed normal also contained ovoid bodies, 20·0–47·5 μ in diameter, together with hyphal bodies 12·0 μ in diameter and about 100·0 μ long. These two conditions often occurred in a cage in which the conidial stage of *E. muscae* had appeared on flies, and they probably represent the resting spore and late mycelial stages, respectively, of the same fungus. In a further 0·9% of flies, from only one sample, the abdomen was distended and a bright orange-red colour between the segments. The abdominal contents of these flies consisted entirely of orange-coloured, spherical, aculeate bodies 37·5–47·5 μ in diameter, probably entomophthorous resting spores. These spores did not germinate on nutrient agar.

Attempts to cultivate *E. muscae* on a wheat extract/peptone agar and egg yolk failed. The fungus sometimes began to grow, but was quickly outgrown by saprophytic fungi and bacteria.

Aphids. Fourteen samples of about 100 pea aphids (*Acyrthosiphon pisum*) on lucerne (*Medicago sativa*) were taken at three sites in October. An

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average of 22% (range 1–46%) were infected with *Entomophthora thaxteriana* Petch and 0·7% (range 0–4%) with *E. aphidis* Hoffman. The largest percentages of infected aphids were associated with crops sown in 1967 and the smallest with the oldest crops sown in 1965.

During August and September many bean aphids (*Aphis fabae*) on field beans at Rothamsted were infected with *E. aphidis*, *E. fresenii* Nowakowski and *E. planchoniana* Cornu.

E. thaxteriana was isolated on sterile egg yolk and on a starch/yeast/peptone agar. It grows and sporulates freely both on these media and on peptone broth. Resting spores occur in egg-yolk cultures.

Endemic infection by *E. fresenii* was maintained on a small percentage of a population of a laboratory culture of bean aphids, but conditions, perhaps humidity, may not have been optimal for the spread of the fungus in the laboratory. *E. thaxteriana* and *E. aphidis* were maintained in the laboratory by exposing healthy pea aphids to spores discharged from diseased ones. Most of those exposed to spores of *E. thaxteriana* died 3 days later when kept at 20–25° C, 4–5 days later at 15° C and 7–8 days later at 10° C. Only very few died after 16 days at 5° C.

It has been suggested that there are different strains of *Entomophthora* spp. adapted to particular host species, but *E. thaxteriana* was readily transmitted from diseased pea aphids to bean aphids.

Preliminary investigations show that few spores of *E. thaxteriana* on pea aphids form below 95% R.H. and that many more form at 100% R.H. than at 95% or even 97% R.H. (Wilding)

Staff and visiting workers

Lesley M. Freeman, P. M. Nuttall and J. Schmid were appointed to the staff. Dr. D. J. Fletcher returned to the University of Natal, and J. Awram (University of Alberta) joined the department as a temporary worker. Dr. Niels Locher of the State Beekeeping Institute, Denmark, spent a week learning our methods of work on the micro-organisms associated with European foulbrood.

L. Bailey was invited to a Colloquium on bee viruses at the Pasteur Institute, Paris, and C. G. Butler to lecture at the Max-Plank Institute for behaviour physiology at Seeweisen and at the University of Frankfurt. J. B. Free who was awarded the D.Sc. degree of London University, attended the 21st International Beekeeping Congress in the U.S.A. and visited several Canadian Universities and Agricultural Research Stations on a lecture tour sponsored jointly by the Nuffield Foundation of England and the National Research Council of Canada.

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