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Rothamsted Report for 1963

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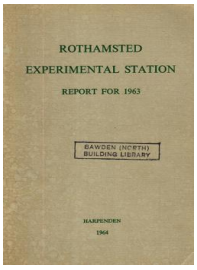

Bee Department

C. G. Butler

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J. P. Spradbery was awarded a Ph.D. degree of London University. Dr. J. Chmurzynski, (from the Nencki Institute of Experimental Biology, Warsaw), spent six months with us working on the behaviour of bumblebees.

Behaviour and Physiology

Queen piping. Many people have supposed that the sound known as "piping", made by queen honeybees, is produced by controlled emission of air through spiracles. It has now been shown that a queen can continue to pipe with all but one of her spiracles blocked. This eliminates the possibility that air is taken in through one set of spiracles and compressed, and sound produced by releasing it steadily through others. Further observations that the pipes are not synchronised with the queen's breathing movements and that the movements continue during the long initial pipe of a series, showed that the sound cannot have been produced by the passage of air through the one unblocked spiracle.

During each pipe a queen vibrates her wings in their folded position. The fundamental frequency of the piping sound is about twice that of the wings in flight, but when a queen held by the legs vibrated her wings without spreading them, or attempted to fly after her wings had been cut short, the frequency was similar to that of piping.

A piping queen presses her thorax to the surface on which she is standing. A cage in which a queen was piping could be felt vibrating when held in the hand, and the sound then was weaker than when the cage was on a bench.

It is concluded that the energy and fundamental frequency of piping are produced by operating the flight motor with the wings folded, that much of the air-borne sound from queen piping in the hive is radiated by the combs and hive timber and that, as bees respond to solid-borne sounds better than to air-borne ones, the pressure of the queen's thorax on the comb enables other bees to hear her better. (Simpson)

Queen pheromones. A honeybee swarm does not form a stable cluster unless it has a queen. Simpson (*Nature, Lond.* (1963), **199**, 94–95) showed that this clustering depends on an odour from the queen's head. Further work shows that a swarm cluster that has begun to disintegrate because its queen has been removed can be re-established and stabilised by the odour of one of the substances, 9-hydroxydec-2-enoic acid, produced in a queen's mandibular glands. Queenless swarm clusters were caused to re-form and remain stabilised for long periods with the odour of this acid.

Although the scent of the queen that attracts workers is also secreted in her mandibular glands, it is not the odour of the 9-oxodecenoic acid nor

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of the 9-hydroxydecenoic acid produced in them, and must be that of another substance or mixture of substances. (Butler with Drs. R. K. Callow and J. R. Chapman, National Institute for Medical Research)

Further work shows that the queen's mandibular glands are the sole source of the sex attractant of the queen honeybee. Experiments with extracts of whole queens, of the contents of their mandibular glands, and with synthetic preparations of several substances secreted in these glands, show that the 9-oxodecenoic acid is responsible for most of the olfactory attraction of a nubile queen to drones. Only the odour of one other substance secreted by these glands, 9-hydroxydecenoic acid, was attractive at all, and it was very much less so than 9-oxodecenoic acid. The odours of these two acids most probably fully account for the olfactory attraction of nubile queens to drones. (Butler and Fairey with Drs. R. K. Callow, J. R. Chapman and N. C. Paton, National Institute for Medical Research)

It has been suggested that drones may be attracted over long distances, even for several miles, by the odour of nubile queens to windward. To test this theory, 2,000 drones were marked in each of three apiaries and, on days when the wind direction was from one of these apiaries towards another, temporary drone catching-points, each supplied with abundant 9-oxodecenoic acid, were set up one at a time at selected positions between these two apiaries. The proportion of drones from the windward and leeward apiaries taken at each catching-point indicated that no long-range upwind attraction was operating, for approximately equal numbers of drones from each apiary were taken at a point mid-way between them. This suggests that drones are attracted only over a short distance, perhaps 10 or 20 yards, by the odour of the queen to windward of them, and that the, often rapid, build-up of drones immediately to leeward of a tethered queen or other source of 9-oxodecenoic acid comes simply from the attraction of individual drones that happen to wander into the relatively small area to leeward of the queen where her odour is strong enough for them to smell.

Other information about drone distribution was obtained by sampling them at various points around apiaries in such isolated places as the East Anglian Fens and over the sea off the Dorset coast. This led to the conclusion that the proportion of drones flying from a particular apiary that can be caught at a given site decreases as the distance of this site from the apiary increases. It is unlikely that many drones fly more than about two miles from their apiaries, and no evidence was obtained of their congregating together in particular places.

Counting drones approaching dead bee lures attached, at various heights above ground, to a vertical string impregnated with 9-oxodecenoic acid, showed that the stronger the wind, the lower the height at which drones fly. At low wind speeds some fly as high as 80 ft or more and most around 50 ft, whereas in strong winds they come down to 10–15 ft from the ground. During a period of calm the drones near a source of 9-oxodecenoic acid become disorientated and soon disperse. (Butler and Fairey)

Temperature regulation. The effect of cold on the metabolism of whole colonies was studied. Honeybee colonies were taken from their hives in

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winter, put with their combs into galvanised iron tanks and exposed to temperatures between 20° and —39° C. Air was drawn through the tanks, and the carbon dioxide it contained was absorbed and weighed. The carbon dioxide production, and hence food consumption, was least at 10° C and increased above and below this temperature. It is clear, therefore, that in winter a honeybee colony controls its cluster temperature partly by varying its heat production. (Free and Simpson)

Pollination and Field Behaviour

Mustard. Mustard was grown in large screen cages, some without insects and others with honeybees. The bees greatly increased the seed yield of the two varieties of white mustard, but not of the brown mustard. Hence white mustard requires insect pollination, and taking honeybees to the crops will probably sometimes increase the yield of seed. (Free and Spencer-Booth)

Sunflowers. The behaviour of honeybees visiting sunflowers was studied. Most visit them for nectar, and when collecting it often become heavily dusted with pollen which is produced in large amounts. A few packed the pollen into their corbiculae, but most discarded it while hovering in the air. Nectar-gatherers usually worked florets in the male stage of development, in which pollen is obtainable, but they often stood on florets in the female stage, in which the stigmatic surfaces are exposed, and so may have pollinated them.

A few bees collected only pollen by scrabbling over male stage florets and scraping it from the anthers, but these bees often changed to collecting nectar as well, and were then more inclined to retain pollen than bees that had never been seen collecting only pollen.

Bees also visited the extrafloral nectaries on the bracts beneath the flower heads and on the uppermost leaves, especially during the afternoon; these bees did not get dusted with pollen, and none visited floral as well as extrafloral nectaries on the same trip.

Pollen-collectors were most active early in the day, probably because the anthers dehisced at this time, and again, to a lesser extent, in late afternoon, when pollen again became available as the growing stigmas forced the rest of the pollen from the anther tubes. (Free)

Fruit trees. The arrangement of honeybee colonies in orchards and their foraging areas were studied. Eighteen colonies of bees were put near the centre of an 11-acre plum orchard and bees counted on trees along four routes radiating from it. During most of the following period the number of bees per tree decreased greatly as distance from the hives increased, but towards the end of flowering the bees became more evenly distributed, probably because most of the nectar and pollen in the flowers of the trees nearest the hives had already been collected.

In each of two years hives of bees were distributed in a 30-acre orchard of dwarf apple trees so that some were in two groups of 9 hives each, each group being in the centre of 9 acres, others in two groups of 4 hives each,

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each group in the centre of 4 acres, and another 4 hives singly, each in the centre of 1 acre. The bees on the groups of trees near the hives and at intervals of 35 yards from them were counted. In general, the numbers of bees were similar at different distances from the hives placed singly or in groups of 4, but decreased with increasing distance from hives in groups of 9.

In another experiment, 3 hives of bees were put at each end of a long, narrow, 4-acre pear orchard. The bees tended to congregate on the trees towards either end of the orchard, particularly in bad weather when few were flying.

All these results support the view that, for efficient pollination, hives of bees are best dispersed in small groups throughout an orchard.

The foraging behaviour of honeybees was studied in an orchard of dwarf apple trees, Cox with every fourth row Grieve. The bees tended to collect pollen only or nectar, sometimes with pollen incidentally. Towards the end of the period most pollen-gatherers changed to collecting only nectar, and a change in the proportion of bees collecting either nectar or pollen in such an orchard probably reflects changes in the behaviour of individual bees.

During any one visit a bee probably worked only 8–10 ft of a single row, and nearly all of the few bees (about 10%) which did move from one row to another went to an adjacent one. Nevertheless, in the course of several days each bee often covered a large area and visited both varieties indiscriminately.

Further observations were made on the effect on fruit set of distance between compatible varieties. In a Cox–Grieve orchard Cox trees adjacent to Grieve had greater initial and final sets than those with rows of Cox on either side of them. In an orchard of dwarf trees of Comice pear, trees with grafts of Conference (every 10th tree) set more fruit than trees without grafts; but, in four other orchards, the fruit set on Conference trees was uninfluenced by proximity to pollinisers and conditions probably favoured effective self-fertilisation, so that yield was not increased by cross-pollination. In a block of Early River's sweet cherry trees, the set was greater in 2 rows adjacent to compatible varieties than in 3 rows not adjacent. Indeed, the sides of trees facing pollinisers set most fruit.

These observations agree with earlier ones (Free, *J. hort. Sci.* (1963), **38**, 129–137). To facilitate even pollination throughout an orchard each main variety tree should have pollinisers on either side of it, and in orchards of dwarf trees pollinisers should be planted in the same rows as the main variety trees. (Free and Spencer-Booth)

The rapid decrease in fruit set with increase in distance from pollinisers probably reflects the small foraging areas of individual bees, but clearly it could be explained equally well by decrease in compatible, air-borne pollen grains if wind pollination were effective. This possibility was therefore examined. The fruit set on small Cox trees in cages was substantial when honeybees were present together with bouquets of a compatible variety, but very little fruit was set when either, or both, the bees or bouquets were excluded. On mature Cox trees flowers from which sepals, petals and stamens were removed, leaving only the stigmas, set little fruit,

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although equally exposed to wind pollination as control flowers. It is probable, therefore, that apple flowers normally require to be pollinated by insects. (Free)

Colony Management

Feeding. When colonies are fed sucrose syrup (usually not much over 60% sucrose w/w) they invert much of the sucrose and evaporate water until over 80% concentration is reached, before sealing the food in storage cells.

It has been suggested that bees could be spared much effort if, instead of syrup, they were fed an invert sugar candy having the same sugar concentration as the final stored product. However, the idea that bees could store such material without needing to evaporate water from it was shown to be mistaken. Although bees feeding on fine-grained candy swallow crystals along with the liquid phase, the material they deposit in the cells is always completely liquid. The honey-stomach contents of bees that had collected loads of candy had an average sugar concentration of about 40%. Thus the bees had added much water (probably from their labial glands) to the candy and would need to evaporate it before storage. The water evaporated would be lost to the bees, which would need to leave the hive and fetch more to be able to continue to collect the candy. Only those bees using the candy for their own immediate consumption could absorb the water from their alimentary canals and re-use it. Thus candy, like dry sugar, is not very suitable for feeding colonies to enable them to store food for winter. (Simpson)

Invert sugar candies prepared by acid-hydrolysis of sucrose and marketed by several manufacturers for feeding to bees were very toxic when fed to caged bees. The time taken for half the bees to die when fed with these candies was 5–6 days, whereas that for bees fed honey, sucrose, 50 : 50 glucose : fructose (laboratory reagent grades) or sucrose inverted with invertase, was at least 18 days. Bees fed the proprietary candies became very dysenteric shortly before they died. Sucrose inverted with organic (tartaric) acid was as harmful as that inverted with mineral (hydrochloric) acid. The mineral in the candies, produced by neutralising the acids after inversion was completed, was too little to account for their toxicity. The darkest of a range of unrefined cane sugars that were tested similarly were also very toxic. (Bailey)

Bee Diseases and Pests

Paralysis. Further differences found between chronic bee-paralysis virus (CBPV) and acute bee-paralysis virus (ABPV) were as follows. CBPV is inactivated by 60% alcohol, whereas ABPV is not. Bees injected with CBPV died sooner at 35° C than at 30° C, whereas those infected with ABPV died sooner at 30° C than at 35° C. Unlike ABPV, CBPV does not survive long in bees' faeces and does not cause disease when injected into adult bumblebees.

Honeybees sent from Hong Kong for diagnosis were suffering from

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chronic paralysis. However, neither CBPV nor ABPV could be found in bees from Australia showing similar symptoms. Nor could ABPV be detected in live, healthy bees from North America, although after these had been established in this country for a few months they were infected with ABPV, like indigenous bees. (Bailey, and Gibbs, Plant Pathology Department)

Sacbrood. Extracts of honeybee larvae suffering from sacbrood contained many isometric particles about 28mμ in diameter. When put in the food of larvae, these extracts or purified preparations of the particles caused sacbrood. The particles were not found in comparable extracts of healthy larvae. They resemble those of acute bee paralysis virus, but infection and serological tests failed to show any relationship between the two viruses. Isolates of sacbrood virus from Europe and North America seemed identical with those from Britain. Of 18 samples of brood sent for diagnosis from various parts of England and Wales, that would previously have been diagnosed as suffering from "addled brood", at least 15 were suffering from sacbrood, as shown by infectivity and serological tests. Thus sacbrood is far commoner in Britain than was previously supposed. Before 1963 the last case diagnosed as sacbrood was in 1935. (Bailey and Gibbs, Plant Pathology Department)

Acarine disease. The queens of several colonies whose bees were infested with *Acarapis woodi* were replaced in spring with mated queens from North America, and the progress of infestation in these colonies was compared with that in adjacent infested colonies headed by local queens. The only striking changes by the end of summer were an increase of bees infested from 20 to 75% in one colony with an American queen and a decrease from 33 to 0% in another. These are changes of an order that have been observed in colonies headed by indigenous queens. All infested colonies appeared normal in autumn. Thus no evidence was obtained of any obvious differences between American and local bees in their susceptibility or resistance to infestation by *A. woodi*. (Bailey and Lee)

Chalkbrood. More than 40% of sealed larvae, whose food had been infected with spores of *Ascosphaera apis*, developed chalkbrood when they were incubated at temperatures below 30° C. Only 12% developed chalkbrood when they were incubated at the normal brood-nest temperature of about 34° C. Thus chilling brood appears to favour development of chalkbrood. This may be connected with the optimum temperature (30° C) for growth *in vitro* of *A. apis*. It may also explain why chalkbrood is commoner in the larvae of drones than in those of workers. Drone larvae are usually on the edge of the brood-nest and are probably more likely than worker larvae to become chilled.

More chilled larvae developed chalkbrood after they had been infected when 3–4 days old than when 0–1 day old. Perhaps the aerobic mycelium of *A. apis* soon dies from a lack of oxygen and unfavourable high temperature in the lumen of the gut of a growing larva. Apart from creating a favourable temperature for the fungus, chilling sealed larvae may allow

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oxygen to diffuse to the gut. Thus the mycelium may be enabled to reach and penetrate the gut wall of a chilled larva before it can start to pupate and void its intestinal contents. (Bailey)

Wax moth. Brood comb sprayed with a suspension of a nuclear polyhedrosis virus obtained from diseased larvae of *Galleria mellonella* was protected against larvae of *G. mellonella* and *Achroia grisella* that developed from eggs laid in it. However, all the polyhedra (about 10^8) from one diseased larva was needed to protect one comb (British Standard Frame) against either species of moth. Lesser amounts of polyhedra (10^4 or 10^6 polyhedra per comb) offered no protection against *A. grisella*, and although they eventually killed all the larvae of *G. mellonella*, they did not prevent the combs being partly or entirely destroyed. (Bailey)

Although combs made from wax foundation containing spores of *Bacillus thuringiensis* are proof against wax moth (see *Rothamsted Report* for 1962, p. 172) it is difficult to disperse spores evenly in foundation in the stages of its manufacture when the wax is cold. Recent tests, however, show that all spores survive at least 80° C in wax for more than 2 hours, so it is hoped to make further trials with foundation made of wax treated while molten, which may be a more convenient industrial method. (Bailey and Dr. D. H. Burges, Pest Infestation Laboratory, Slough)

Eggs of *Galleria mellonella* and of *Achroia grisella*, laid in combs, were killed within 24 hours exposed to propylene oxide in the way found successful for killing adults and larvae as well as dormant stage of several honeybee pathogens (*Rothamsted Report* for 1962). Tests against *Bacillus larvae* spores in boxes full of combs were less satisfactory than they were against spores in empty containers, however, which suggests that wood and wax absorb much of the fumigant. Honey exposed to the fumigant did not become toxic to bees. (Bailey and Lee)

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