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ROTHAMSTED RESEARCH

Report for 1964

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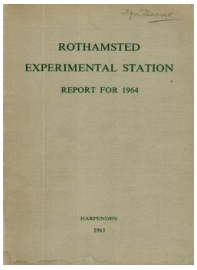

Bee Department

C. G. Butler

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BEE DEPARTMENT

C. G. BUTLER

D. C. Lee left to take an appointment at the South Australian Museum and D. C. Bowler was appointed.

Several members of the Department contributed to the 12th International Congress of Entomology in London in July.

Behaviour and Physiology

Queen piping. Honeybee colonies with adequate hive space rarely swarm until they have one or more young queens ready to emerge from their cells. For a day or two before swarming the worker bees keep the young queens from fighting by preventing them from emerging, and during this confinement the queens in the cells and the free queen both make sounds known as piping. Whether queen piping plays a part in making a colony swarm was studied with an apparatus devised to feed recorded sound into the woodwork of a hive. (Bees can only perceive sounds communicated by their substratum.)

During 10 days while recordings of queens piping were fed continuously into an observation hive containing a large colony, no swarming occurred. When the sound was turned on again, 6 days after the old queen had died and been replaced by one young virgin queen, a swarm left the hive but returned as the queen had not accompanied it. The next day a swarm of bees left the hive with the queen. During the period between the two swarmings the young queen remained continuously on a comb where several queens had recently been reared, frequently piping and examining the remains of the cells from which the queens had emerged. She appeared to be searching for the queen producing the recorded piping and to be attracted to the remains of the queen cells by traces of queen scent on them. The act of destroying queens before they emerge from their cells usually indicates that a colony is not going to swarm, so this colony would probably not have swarmed had the piping sounds not been played to it, but it is clear that piping alone does not make a colony swarm. (Simpson)

Attempts to make uncrowded colonies swarm. Uncrowded colonies swarm only when they rear queens because queen substance is insufficient, but many colonies that do rear queens nevertheless fail to swarm. A large colony containing a high proportion of young bees is commonly supposed to favour swarming, and to test this idea many young bees were added to experimental colonies, which were dequeened to make the bees rear emergency queens. However, when the young queens matured these colonies neither swarmed nor did the worker bees confine any of the young queens in their cells. The young queens produced by a colony that has been dequeened are usually all about the same age, unlike the queens

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reared by a colony that has a laying queen, so other experiments were made in which each colony was given a series of queen cells of different ages to simulate queen rearing in colonies with laying queens. Even then, workers did not confine any of the queens in their cells or swarm. Further experiments with very large colonies are planned. (Simpson)

Previous work showed that colonies in small hives swarm because hive space is too little for adult bees, not because space for brood is inadequate. This removed a misconception that has greatly confused the swarming problem for at least half a century. Indeed, although details have still to be explained, particularly concerning the reluctance of worker bees to pass through queen-excluders or sometimes to occupy empty space, it should soon be possible to give advice that will enable beekeepers to avoid completely what is probably the commonest cause of swarming, namely, insufficient hive space.

However, this will not solve all management problems, because swarming is probably less damaging than inefficient queen replacement ("supersedure"), which is frequent in colonies that do not swarm and leaves many colonies without laying queens for long periods. Therefore the main practical problem may be of preventing unnecessary queen rearing rather than of preventing swarming, so there is good reason to hope that this will be solved when more of the pheromones in the queen substance complex, by which good-quality queens inhibit queen rearing by the workers, have been identified and synthesised. This may also be solved in other ways than supplying colonies with synthetic pheromones, for it seems unlikely that honeybees have naturally evolved such a harmful piece of behaviour as inefficient queen replacement, particularly as many colonies do replace their queens efficiently with no break in oviposition. By breeding from colonies that do not swarm when greatly congested, beekeepers may have selected strains in which queen rearing is normal but the swarming mechanism defective, or in which the mechanism fails when these strains are kept in environments different from those in which they evolved. A better strain of non-swarming bees might be produced by breeding for queens that can inhibit queen rearing in much larger colonies than their egg-laying will produce. The inhibitory powers of queens could perhaps be tested by putting them for short periods into very large colonies made by dequeening and uniting several large ones.

Queen pheromones. Queen honeybees produce an odour, attractive to worker bees, which persists in dead queens for several months and can be extracted in ether and some other organic solvents. It is soon lost when such extracts are exposed to the air. This attractive odour is neither that of 9-oxodecenoic acid, which together with an unidentified adjuvant substance inhibits queen rearing, nor that of 9-hydroxydecenoic acid, which stabilises the swarm cluster and, like 9-oxodecenoic acid, is produced in a queen's mandibular glands. We have confirmed that excising a queen's mandibular glands removed most, but not all, of her attractive scent. Our results indicate that the other source of her attractive scent lies in her abdomen, although traces remained in her head ten days after her mandibular glands had been removed as completely as possible. Further

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experiments showed that the attractive scent does not come from the queen's subepidermal glands, the ducts of some of which open on to the intersegmental membranes between the abdominal tergites and others on to the dorsal surfaces of these tergites. Possibly the secretion of these glands is an aphrodisiac that stimulates the drone when, attracted by the odour of 9-oxodecenoic acid, he intercepts and approaches closely a flying, nubile queen. The second source of the scent that attracts workers comes from the paired Koschewnikow glands, whose ducts open into the queen's sting-chamber. (Butler and Simpson)

Some progress was made, in collaboration with Drs. R. K. Callow and J. R. Chapman (National Institute for Medical Research), in isolating and identifying the queen's attractive scent for workers.

Queen rearing in colonies of the Western honeybee (*Apis mellifera*) is inhibited by queen substance (i.e., 9-oxodedenoic acid + inhibitory scent). It seems unlikely that this phenomenon is specific to *Apis mellifera*, and queens of other species of honeybees and perhaps of other social insects probably produce similar, or identical, substances with comparable biological action. Extracts of the whole queens (i.e., queen substance) of the Eastern honeybee (*Apis cerana* var. *indica*), of the Little honeybee (*Apis florea*), and of the queens of the termites, *Odontotermes* sp. and *Zootermopsis angusticollis*, of the bumblebees, *Bombus terrestris* and *Bombus pratorum*, of the social wasp, *Vespula germanica*, and of the ants, *Formica fusca* and *Myrmica rubra*, were tested for biological activity on queenless workers of *Apis mellifera*. (Unfortunately queens of the other species of honeybee, *Apis dorsata*, were not available.) The results showed that ethanol extracts of *A. cerana* queens inhibited queen rearing as strongly, or nearly so, as did extracts of *A. mellifera* queens. Extracts of *A. florea* queens were probably less effective, particularly in inhibiting ovary development. Slight inhibition of both kinds was obtained with extracts of the queen of one of the species of termite, *Odontotermes* sp., and of the ant, *Formica fusca*. Extracts of the queens of the bumblebees, of the wasp, and of the other species of termite and ant had very little, if any, effect. These results suggest that the two closely related honeybees, *A. mellifera* and *A. cerana*, share a queen substance, which not only inhibits queen rearing by workers of both species but also ovary development in their workers. The third honeybee examined, *A. florea*, also seems to have a similar queen substance. It will be interesting to discover whether 9-oxodecenoic acid is the most important component of the queen substances of *A. cerana* and *A. florea*, and, if so, whether it attracts their drones during nuptial flights and is the only sex attractant, as it is in *A. mellifera*. (Butler)

Hypopharyngeal glands. The hypopharyngeal glands of worker honeybees produce enzymes used in converting nectar into honey and also secrete the protein portion of the food fed to larvae. Workers that have become foragers usually have smaller glands than workers that are still housebees, but, nevertheless, their glands contain invertase. We have found that invertase becomes abundant before the glands decrease in size, but is still plentiful in glands so shrunken that their secretory cells contain little

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cytoplasm. When bees fill their crops (honey-stomachs) with food they add to it about as much invertase as their glands contain. These facts suggest that, even after these glands seem to have atrophied, they continue to secrete invertase rather than withdrawing it from some reserve.

Bees 7 and 14 days old had very little invertase in their glands during June and July, but much by August and September. The glands of bees 21 and 28 days old were larger in August and September than in June and July. The glands of bees in winter remained large despite the bees' age, but contained much invertase; this was true even of bees feeding brood, which in summer have little invertase. Thus in the later part of the summer colonies apparently undergo physiological changes leading to the winter condition. Whether these changes are determined within the colony or by the environment remains to be determined. (Simpson and Riedel)

Pollination and Field Behaviour

Stimulation by feeding syrup. Feeding colonies with sugar syrup stimulates their bees to collect more pollen than usual. It does so by causing many of the foragers to forsake nectar-gathering in favour of pollen-gathering. Because pollen-gathering bees are more efficient pollinating agents than nectar-gathering bees, it seemed probable that feeding colonies with syrup might be useful when they were being used to pollinate particular crops. Before such feeding could be recommended, however, it was desirable to find out whether a colony that was moved to a particular crop would, when stimulated in this way, collect more pollen from this crop. Two groups of colonies were therefore taken to crops of sweet cherry, field beans and red clover, and one group was fed sugar syrup continuously and the other not. Syrup feeding increased the total amount of pollen collected by about 3·5 times, and the proportion collected from the crops requiring pollination was at least as great as that collected by the unfed bees. (Free)

It has been suggested that honeybees are encouraged to visit particular crops by spraying them with sugar syrup and that this will give more effective pollination. This suggestion was tested by spraying plots of field beans, and also apple trees, with sugar syrup. This greatly increased the number of bees visiting the sprayed areas of these crops, but, if anything, it decreased pollination, because some of the bees that had previously been collecting pollen from the flowers changed to nectar-gathering. Further, the stamens of the apple flowers became too sticky with syrup for bees to gather pollen. These factors resulted in less fruit being set in the sprayed than in the unsprayed plots. (Free)

Fruit trees. The pollinating value of honeybee visits to apple flowers was studied. Clusters of flowers were covered with plastic bags when in bud; later, the newly opened flowers were freely exposed and watched continuously until a honeybee visited them. The number of flowers in a cluster that a bee visited and its behaviour were recorded. The flower cluster was covered again as soon as the bee left it. Bees that scrabbled over the flowers collecting pollen set more fruit than those that collected

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nectar, and a greater percentage of the visits paid by bees to flowers of James Grieve (a variety in which self-pollination is effective) than to flowers of Cox's Orange Pippin (a variety requiring cross-pollination), resulted in fruit being set. The flowers of Cox's growing in rows adjacent to rows of Grieve's gave a greater set when visited than the flowers of Cox's surrounded by other Cox's, confirming the results of previous observations on the effect of the limited foraging areas of individual bees on fruit set. (Free)

When foraging honeybees leave their hives it is possible they still carry viable pollen collected on previous foraging expeditions on their bodies. To test this possibility, small Cox's trees were caged. Some were caged with a honeybee colony only, others with a honeybee colony and a bouquet of flowers of a compatible variety of apple, others without a honeybee colony or bouquet. Each cage of this last group had about 20 foragers that were about to leave their hives directed into it every half-hour during much of the flowering period. No fruit was set in those cages containing honeybee colonies but no bouquets, but plenty of fruit set in the cages belonging to the other two groups, showing that when bees leave their hives they sometimes carry viable pollen on their bodies and, indeed, the pollen carried in this experiment was not only viable but set fruit on Cox's. (Free)

Blackcurrants. Blackcurrants benefit from insect pollination, and it has been suggested that yields might be increased by cross-pollination between different varieties. If this is so, the fruit set should be greatest on those bushes growing next to other varieties and should decrease with increase in distance from other varieties. In 1963 and 1964 blackcurrant plantations containing blocks of two or more varieties were examined, and the fruit set on bushes growing in rows adjacent to another variety was measured and compared with that on bushes farther away from this variety. The distance a bush of one variety was away from one of another variety had no measurable effect on the quantity of fruit set, indicating that the varieties concerned (Baldwin, Cotswold Cross, Davison, Mendip Cross, Seabrooks, Wellington XXX, Westwick Choice) did not benefit from cross-pollination. (Free)

Sunflowers. The pollination requirements of sunflowers of the variety Pole Star were studied by bagging the heads singly and in pairs, with and without bumblebees. Heads without bumblebees produced few, or no, seeds. This suggests that when, towards the end of flowering, the stigmatic lobes of a floret curl downwards, any of its own pollen they touch is ineffective. A larger percentage of the florets set seed when two heads were caged with bumblebees than when single heads were caged, showing that cross-pollination is more effective than self-pollination with this variety. (Free and Simpson)

Broad beans. The seed yields of broad bean plants caged with and without honeybees were compared. Plants without bees set a considerable number of seeds, but those with bees produced more and bigger seeds per

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pod and, in particular, set a greater proportion of the earlier flowers, thus producing an earlier crop. (Free)

Use of solitary bees as pollinating agents. The females of many species of solitary bees characteristically excavate their nest-burrows close together in severely restricted parts of an apparently suitable terrain. Some of these restricted nesting-sites persist for many years with little or no change in position or area, and some of the solitary bees that behave in this way are valuable pollinating agents. One such is *Andrena flavipes*, a frequent visitor to flowers of early fruit trees. A large nesting-area of this species contains thousands of individuals, making up a powerful pollinating force, so it is worth while considering the possibility of establishing new "colonies" in fruit-growing areas. Before this is attempted, however, it is desirable to study their behaviour and ecology and to find the reasons for the "colonies" being so compact and for some places being preferred to others.

Observations showed that *Andrena flavipes* nests in banks of sandy loam, the surfaces of which erode enough to keep them almost free from vegetation. The males patrol the nesting-areas and parts of the banks surrounding them in search of nubile females basking in the sun on the ground. Experiments showed that a patrolling male recognises a female by the bright orange-coloured hairs on her hind-legs and will try to mate with models, provided these are about the right size, have orange-coloured hind-legs and, most important, are situated either in, or very near to, an established nesting-site. Further experiments showed that a male will approach and attempt to mate with a female, or suitable model, only when she is associated with an odour coming from an occupied nesting-site. Also, nubile females seldom bask on the ground outside this area, and only when overcrowding is extreme do they excavate nest-burrows outside it. When soil from a nesting-site was removed to another place near by and dummy females placed on it, some of the males that flew over it alighted on or beside the dummies and attempted to copulate with them; further, a few mated females from the original nesting-site that found the "new" site nested in it and so established a new nesting-site.

An attempt to establish a new nesting-site several miles away from a well-established one was successful for two years, but failed in the third because grass and other plants grew over its surface. (Butler)

Bee Diseases and Pests

Paralysis. Each of several samples of bees from Austria and Switzerland, suffering from so-called "Waldtrachtkrankheit", and of bees from Italy and Norway, suffering from a disease of similar appearance called "Mal Noir" or "Schwarzsucht", contained as much chronic bee-paralysis virus (CBPV) as bees with "paralysis" in Britain. These diseases, therefore, appear to be etiologically the same.

Apparently healthy bees from North America and Italy were infected with acute paralysis virus (ABPV), as they are in Britain.

ABPV did not increase in local bees as these aged; nor was there more in bees that died naturally than in live bees of the same age. There is no

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evidence, therefore, that natural infection with the virus causes premature death.

Antisera prepared against ABPV or CBPV neutralised much of the infectivity of either virus, but the two contain specific in addition to common antibodies because the infectivity of each was still neutralised by its homologous antiserum after this had been fully absorbed with the other virus. (Bailey)

The signs of paralysis suggest damage to nervous tissue and could be confused with those of poisoning by insecticides, but cholinesterase activity of bees with either acute or chronic paralysis was equal to that of normal bees. Paralysis should not therefore be mistakenly diagnosed as damage by organophosphorus insecticides when these are detected by decreased cholinesterase activity. (Bailey and Stevenson, Insecticides Department)

Sacbrood. When whole larvae infected with sacbrood virus were kept at 18° C after they died infectivity was undetectable after 3 weeks, whereas thin smears of similar larvae or of their ecdysial fluid, allowed to dry at the same temperature, were as infective after 3 weeks as in newly diseased larvae. Such smears lost their infectivity within 10 months, and the natural means of transmission of sacbrood, which usually clears up spontaneously by mid-summer, remains unexplained. (Bailey)

Dysentery. The size and rate of growth of colonies in the spring of 1963 was negatively and significantly correlated with the percentage of bees naturally infected with *Nosema apis*. This was not so in 1964, and the correlation may be only indirect, acting through "dysentery", which spreads *Nosema apis* and, in a severe form, was the only obvious common factor in the many colonies that died in the winter of 1962–63. Dysentery, therefore, or unknown related factors, are possibly more important causes of small size and poor growth of colonies in spring than *Nosema apis*, which has usually been held responsible.

Acid-inverted sucrose, which causes dysentery and death when fed to bees (*Rothamsted Report* for 1963, p. 165), contains 5-hydroxymethylfurfuraldehyde (HMF). More than about 0·2% of HMF, or of laevulinic acid, which forms spontaneously from HMF, causes dysentery and death when fed in sucrose syrup to bees. The invert sugar candies produced by several manufacturers from acid-inverted sucrose has the same effect on bees as 1% HMF in sucrose. HMF, or its related compounds, may sometimes be significant in nature, as it occurs, usually in very small amounts, in honey. (Bailey)

European Foulbrood. Two strains of *Streptococcus pluton* isolated from diseased larvae of *Apis mellifera adansonii* sent from Tanganyika did not differ serologically from any of the many strains isolated from European or American honeybees. The appearance of the colonies of one of them on agar seemed unusual, however, being grey and sometimes ring-shaped instead of white and raised. Otherwise, the cultural characteristics of the African strains entirely resembled all those isolated from elsewhere. (Bailey and Gibbs, Plant Pathology)

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Chalkbrood. Larvae fed spores of *Ascosphaera apis* and then chilled for as little as 6 hours at about 20° C when they had been sealed in their brood comb cells developed chalkbrood after they were returned to their parent colony. Uninfected larvae developed normally after the same treatment, as also did infected larvae incubated for the same length of time but at the usual brood temperature of about 35° C. Before treatment the gut contents of infected larvae contained many spherical mycelia about 30–50 μ in diameter, and these appeared unchanged after the chilling. Thus, chilling does not necessarily cause chalkbrood by providing an optimal temperature for the fungus, but must cause effects that later activate mycelial growth at usual brood temperature.

Beekeeping practices that cause heat loss can be expected to increase the incidence of chalkbrood in colonies that are endemically infected with *Ascosphaera apis*. Severe disease was observed in private apiaries where most of the brood of colonies had been routinely separated from the queen in early summer with the intention of preventing swarming. This practice spreads the cluster of bees and probably causes heat loss. (Bailey)

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