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ROTHAMSTED RESEARCH

Report for 1970 - Part1

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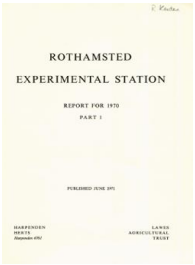

Bee Department

C. G. Butler

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BEE DEPARTMENT

C. G. BUTLER

The practical aims of the department are to lessen losses from pests and diseases, to improve bee-husbandry practices and to enhance the value of bees both as pollinators and as honey producers.

Work has continued in attempts to discover the factors determining the behaviour of honeybees in both the hive and the field, and the conditions in which pathogens become damaging. The spread of diseases among some pest insects was also studied.

Behaviour and physiology

Swarming. Beekeepers have found that colonies of the Western honeybee (*Apis mellifera* L.) rarely swarm unless they contain occupied queen cells, and many believe that a colony's first swarm usually leaves the hive with the old mother queen about the time that the first queen cells are sealed. In contrast to these beliefs, large colonies put experimentally into small hives have swarmed before starting to rear queens, whereas colonies with more than adequate hive space have swarmed only after young queens have been reared and have killed the old ones. This year we put colonies into hives that, although small, were large enough for the bees at the time, and then allowed the colonies to outgrow the hives. These colonies swarmed with their old queens, and only after they contained sealed queen cells or at least queen cells containing larvae. Probably, therefore, the traditional picture of swarming behaviour has been drawn from colonies with too little hive space. Unfortunately it is not yet clear whether crowding adult bees encouraged queen rearing in addition to swarming, or whether queen rearing made congested colonies more ready to swarm. However, the observations suggest that colonies allowed to outgrow small hives may be suitable material for testing the effectiveness of the common beekeeping practice of removing queen cells to prevent swarming, a test almost impossible to make with unrestricted colonies because those with queen cells swarm so rarely. (Simpson and Moxley)

Queen-rearing temperatures. Worker larvae placed in artificial, wax, queen-cell cups are more readily accepted and a greater proportion survive when they are in the middle of a rearing colony than when they are at its periphery. The reason may be the more uniform and warmer temperature at the centre. Thermocouples fitted close beside the queen cells showed that whereas the temperature at the periphery and the centre differed by no more than 1°C during warm weather, it differed by 3°C during cool weather, despite the fact that the rearing colony was so congested in its hive that some bees had to cluster outside the entrance. (Simpson and Moxley)

Hoarding behaviour. The food-hoarding behaviour of honeybees was studied using groups of 50 workers in small cages, each of which was provided with a piece of comb and gravity feeders containing sucrose syrup and distilled water, respectively. Less of the syrup was stored in new than in old comb previously occupied by brood or pollen, and less in worker than in drone comb. Presence of syrup in the comb made no difference to the amount later stored in it. Increasing the area of comb from 22 to 90 sq cm did not affect the proportion of cells in which syrup was stored, or the amount of syrup stored. The amount stored increased with increase in environmental temperature from 25 to 35°C.

Putting a caged queen among the small groups of bees induced them to store more syrup, but the odour only of a queen did not. The presence, or odour only, of larvae decreased the amount of syrup stored.

The amount of food stored also depended on the age and experience of the bees. Those that had been foragers stored more than those that had been house bees. Bees that had been deprived of food, even for only a short period, subsequently kept more syrup in their honeystomachs, but did not store more in the combs, than those given continuous access to syrup. Bees compensated for the absence of comb, or of the more favoured kinds of comb, by keeping more syrup in their honeystomachs. (Free and Williams)

Pollen collection. Honeybee colonies were fed pollen (taken from a pollen trap at the entrance of a hive), pollen supplement (i.e. 1 part dried brewer's yeast + 4 parts soya bean flour mixed together with sufficient sucrose syrup to make a stiff paste), or a mixture of 5 parts of pollen supplement + 1 part of pollen, in Petri dishes immediately above their brood combs. Foragers of colonies fed pollen collected less pollen than foragers of unfed control colonies, whereas feeding pollen supplement, or the pollen supplement and pollen mixture, had no effect on pollen collection. Therefore, it is improbable that the practice of feeding existing pollen supplements to colonies that are being used to pollinate crops diminishes the numbers of pollen-gatherers, which are usually more valuable than nectar-gatherers as pollinators. Nor would it be wasteful to supplement the natural pollen available to foragers. Attempts to increase pollen collection, by having pollen traps at the entrance of hives to prevent some of the collected pollen entering the colonies, failed. (Free and Williams)

Bumblebee flight paths. Males of many species of bumblebees follow regular flight paths between various objects, such as leaves or twigs, that they mark with pheromones secreted by their mandibular glands. Queen bumblebees also visit these marked objects, so facilitating mating. The attraction of males of *Bombus pratorum* to queens that visit their flight paths was studied, using queens that were suspended in the air by black thread attached round their waists. Whereas males usually reacted to queens suspended near objects marked with pheromone, they usually ignored queens that were suspended along the flight path but further away from scent-marked objects. The black colour of a queen attracted males but the orange or yellow bands on her body did not; nor did movement by the queen. Her larger size made a queen more attractive than a worker to males. Her odour was also important both in inducing males to seize her and in enabling them to distinguish between queens of their own and other species of *Bombus*. Males made more attempts to mate with virgin queens than with older, mated, laying queens; perhaps changes in odour associated with age or ovary development were responsible. (Free)

Pheromones of queen honeybees. Many queens whose mandibular glands have been removed can still inhibit queen rearing by their colonies, so a pheromone, or pheromones, additional to those in their mandibular gland secretion has been suspected to have this action. However, proof was difficult because it was difficult to be certain that these glands were completely removed and that none of their secretion remained. New evidence for complete removal was obtained by chemical analysis and biologically; the biological test was with queens that had been returned to their colonies after removing their mandibular glands and keeping them there so that the bees removed any residual secretion. Such queens no longer attracted drones from a distance as do normal queens by the odour of the 9-oxodec-*trans*-2-enoic acid produced in their mandibular glands, which now seem the only ones that secrete it. Therefore, this unidentified inhibitory pheromone

seems to be produced by other glands of the queen. Perhaps it is the inhibitory scent Butler (*J. Insect Physiol.* (1961), **7**, 258–264) found was produced by queens without mandibular glands. (Butler, with Callow, Insecticides Department)

Results last year (*Rothamsted Report for 1969*, Part 1, 256) strongly suggested that, in addition to 9-oxodecenoic acid, a queen's abdomen at the time of her nuptial flight carries another olfactory, or possibly gustatory aphrodisiac. Further experiments on mating behaviour confirmed this; queens whose mandibular glands had been completed removed were tethered 6 m above the ground and synthetic sex attractant (9-oxodecenoic acid) exposed near them to attract free-flying drones from a distance. Close examination of such a queen by a drone was followed either by his flying away or by his seizing the queen from behind with his forelegs round her abdominal segments V and/or VI and vigorously palpating her abdominal tergites with his antennae. Palpation was followed either by the drone releasing the queen or curling his abdomen round and attempting copulation. Each of these responses was stimulated by an unidentified substance perceived by the drone on the queen's abdominal tergites; obviously this substance must be produced in other than the mandibular glands, perhaps those described by M. Renner and M. Baumann in 1964 (*Naturwissenschaften* **51**, 68–69). (Butler)

A queen honeybee examines the interior of a cell in a comb before turning and laying an egg in it; she then moves away without paying further attention to the cell, which is soon entered by a worker bee. Tests were made to find whether a queen deposits mandibular gland secretion, containing 9-oxodecenoic acid, when inspecting a cell before laying in it, thus supplying the pheromones contained in this secretion to the worker who enters the cell. Empty brood cells, cells in which a queen had laid but which no worker had yet entered, and cells workers had entered after a queen had laid in them, were washed out with methanol to extract any 9-oxodecenoic acid they contained. None was found in any of the cell washings, but there was 10-hydroxydecenoic acid in the washings of cells workers had entered immediately after a queen had laid in them. 10-hydroxydecenoic acid is produced in the worker bee's mandibular glands and forms a major part of the 'brood food' she gives to larvae. What significance there is in workers putting some of this glandular food near or on an egg as soon as it is laid is being examined. (Butler and Koster, with Callow, Insecticides Department)

The stimuli causing worker honeybees to form a 'court' round their queen and to examine her with their antennae were sought. Velthuis and van Es (*J. apicult. Res* (1964), **3**, 11–16) reported that queens without mandibular glands can elicit apparently normal 'courtship' behaviour, and we confirmed this using queens that, when tested chemically and biologically, seemed to be free from residual mandibular secretion. Extracts of these queens elicited courtship behaviour, as did the mandibular gland secretion of entire queens. Therefore, the unidentified pheromones that attract worker bees probably occur both in the mandibular glands and other parts of queens. (Butler and Koster, with Callow, Insecticides Department)

Field behaviour

Pollination. Attempts are sometimes made to increase the set of tree fruits by applying pollen to some of the flowers artificially. Success would be greater if bees dispersed this pollen among the other flowers. To discover the extent of such dispersal, pollen was applied by hand to many clusters of flowers, during 1965 and 1970, in apple, pear, plum and sweet cherry orchards where honeybees were kept. The clusters of flowers chosen were 5 m apart, and the set of other flowers between 0·3 and 2·5 m away from them was later measured. Although the hand-pollinated flowers usually set more fruit than the

others, the flowers nearest to them set no more fruit than those furthest away. It seems, therefore, that the bees did not distribute the artificially applied pollen to any great extent, but repeat tests are needed with larger quantities of pollen. (Free and Williams)

To be suitable for exploiting as a pollinator, any species of solitary bee must fulfill most of the following criteria: (a) readily able to occupy artificial nests, such as drinking straws; (b) nest gregariously; (c) able to develop a large population quickly; (d) forage preferentially on the flowers of the crop it is required to pollinate. As a first step in discovering whether any of the local solitary bees are suitable as pollinators, artificial nests, consisting of bundles of drinking straws protected from rain, have been exposed in several places during each of the last 5 years. The solitary bee, *Osmia rufa*, was the most frequent occupier of these straws, but sometimes there were others, such as *Osmia coerulescens* and *Megachile* spp., and a few solitary wasps, *Ancistrocerus* spp. The number of straws occupied differed much in different places and probably reflected the size of the local population of these bees. *Osmia rufa* collected most of its pollen from buttercups (*Ranunculus* spp.) and from the oak, *Quercus robur*, and it seems probable that the local abundance of these plants partly determines the number of bees of this species that are present. There was no evidence that a shortage of natural nesting sites limited population at any of the places studied.

After the first year, a few straws that already contained nests were added to every bundle exposed at each site. At the most favoured site, *O. rufa* tended to nest gregariously and the initial population increased from two to seven times in different years. However, at most sites, the average number of straws occupied did not increase during the year. *O. rufa* seemed to be attracted to bundles of straws that already contained a few occupied ones and straws that had previously contained nests were preferred to clean ones. *O. coerulescens* also behaved in this way, but *Ancistrocerus* spp. did not. *Megachile* spp. were too few for any conclusions. (Free and Williams)

Bee diseases

Paralysis. Chronic bee-paralysis virus was readily detected, by infectivity and serological tests, in live bees taken during autumn and winter from seemingly healthy colonies at Rothamsted with no history of paralysis. Late in autumn most individuals contained the virus, which was recovered from their salivary and hypopharyngeal glands. The proportion of bees infected in these colonies was the same as in colonies headed for long by queens reared from paralytic colonies. The virus was not readily detected in live apparently healthy bees from the same colonies during summer, although it occurred commonly in similar bees from the few colonies with overt signs of paralysis. By contrast, acute bee-paralysis virus could not easily be found during winter, whereas most bees in summer were infected. Both viruses were detected frequently in pollen loads of foraging bees. Significantly more chronic paralysis virus was in pollen collected by bees of apparently healthy colonies headed by queens reared from paralytic colonies, than in pollen of bees from normal colonies. The pollen collected by bees from colonies that were losing many bees from paralysis contained much virus.

Acute paralysis virus was recovered from the thoracic glands of apparently healthy bees that also had the virus in their abdomens but not in their heads. Few similar bees at any time during summer had acute paralysis virus in their heads but most had it in their abdomens. By contrast, most apparently healthy bees infected with chronic paralysis virus had it in their heads. All this suggests that inapparent infection by acute paralysis virus is of the thoracic glands, whereas similar infection by chronic bee-paralysis is also of the head glands. There is much evidence that susceptibility to paralysis is genetically

determined, although tests show that few apparently healthy workers, even from colonies losing many bees from paralysis, are susceptible when fed chronic paralysis virus. The hereditary factors are probably recessive, otherwise the queens transmitting them would not survive as long as they do. However, drones are produced from unfertilised eggs so they should express recessive genes and, were only one or two genes involved, the queen of a paralytic colony should produce many susceptible drones. On the contrary most newly emerged drones from paralytic colonies, as from elsewhere, were not susceptible except when fed very much paralysis virus. Drone larvae also from paralytic colonies all produced normal adults when fed much paralysis virus. Susceptibility may, therefore, be multifactorial with few chances of the many genes required coming together. An alternative explanation, based on evidence from elsewhere, is that susceptibility is matroclinous, but if it is, then susceptibility must be transmitted very sporadically. (Bailey)

Sacbrood. Although old bees are immune to infection when fed sacbrood virus, bees from colonies that have been broodless and queenless for several weeks are susceptible, probably because they have physiological characteristics of young bees. A small colony, composed entirely of such bees fed with sacbrood virus, was established with a normal laying queen. In contrast to young bees similarly infected, they reared larvae, about 18% of which died of sacbrood. It seemed improbable that the adult bees had infected the larvae mechanically, because after feeding with sacbrood virus they were kept caged in an incubator for 3 days before they were put into their hive, and previous experiments showed that old immune bees treated similarly were not infective after 2 days. The infected bees, now dwindling rapidly in numbers, then reared a second batch of brood, of which the youngest larvae hatched 13–16 days after the bees had been fed on sacbrood virus. However, only three out of a total of 46 of these larvae developed sacbrood, and all of a further 47 that hatched up to five days later were healthy. The decreasing ability of bees to transmit sacbrood may have reflected the death of individuals in which virus had multiplied most.

Bees from a colony composed of young individuals that had been fed sacbrood virus between 5 and 10 days previously were caught on their return from foraging for pollen and their pollen loads removed. Extracts of the pollen were injected into drones and sacbrood virus multiplied in them enough to be detected easily by serological methods (see *Rothamsted Report for 1969*, Part 1, 259). Comparison with previous infectivity tests suggests that each pollen load contained about 10^6 particles of sacbrood virus, which was good evidence that the infected bees secreted the virus from their glands into the fluid they add to pollen they gather. Infected adults probably similarly infect larvae, but proof that adult bees can transmit sacbrood virus to larvae will be lacking till bees injected with the virus are shown to do so. (Bailey)

The bees in colonies composed entirely of young individuals infected with sacbrood clustered more tightly than usual, even when their hive was artificially heated, and often they gathered outside in the sunlight on hot days. They probably felt chilled, because young bees infected with sacbrood virus became immobile at 0°C sooner than uninfected bees. When small colonies were formed of young bees, of which only some were infected with sacbrood virus and marked, most of the uninfected bees collected on the periphery of the cluster, and only they attended the queen.

The histology of larvae infected with sacbrood virus seemed normal until the prepupal stage. The endocuticle of the last larval skin was shed normally, although it then remained undissolved to form the sac, and the new pupal cuticle formed normally beneath. There was, therefore, no evidence that sacbrood virus affects the production of juvenile and moulting hormones, as has been suggested. The effect of the virus on the epidermis may

be only to inhibit the production of enzymes that dissolve endocuticle. Instead of this enzyme the epidermis presumably secretes the virus that occurs in the ecdysial fluid of larvae with sacbrood. Other histological abnormalities of prepupae with sacbrood were many basophilic granules in the epidermal cytoplasm and failure of the fat cells to disintegrate. (Fernando)

Pathology of other insects

Entomophthoraceae

Field incidence in Wheat Bulb fly. One hundred Wheat Bulb flies were captured each week, as in previous years, from 24 June to 11 September in Stackyard field, to estimate the incidence of fungal infection. By contrast with the last three years, many flies were infected with *Entomophthora muscae*. The percentage that died infected in the first sample was 19% of males and 34% of females; the maximum, recorded two weeks later, was 67% of males and 81% of females. As the peak emergence of flies was about 22 June and the first eggs are usually laid at least a month after emergence, many females were killed before they had laid any eggs. Most infected flies, and all the males, produced conidiophores, but resting spores developed in 25% of infected females. Similarly, in flies infected experimentally, resting spores developed only in females. Resting spores formed in some of the females captured each week from 7 July to 29 July inclusive and the ratio of infected females with resting spores to those with conidiophores increased 1 : 10 to 1 : 1 during that period. *Entomophthora* species probably overwinter as resting spores and the factors that induce the fungus to form resting spores were already operating early in July, and became more evident later in the month.

Many Wheat Bulb flies infected with *E. muscae* were also found at Edelsborough (Bedfordshire), Barton (Bedfordshire) and Earith (Cambridgeshire) but none was found anywhere infected with other *Entomophthora* species.

Field incidence in aphids. Two samples of 100 adult apterous pea aphids (*Acyrthosiphon pisum*) were taken each week from 16 June to 18 August from lucerne on Highfield to estimate the incidence of *Entomophthora* spp. Infected aphids were not found until 1 July, when the percentage infected with *E. thaxteriana* rose from 2% to a peak of 40% on 27 July, and with *E. planchoniana* (first recorded, last year, on one pea aphid after three seasons of sampling at Rothamsted) rose from 1% on 13 July to 16% on 21 July. *E. aphidis*, common on pea aphids in 1968 but not found in 1969, was again found but in only a few aphids at the end of July and beginning of August. The percentage of aphids infected by all three species of *Entomophthora* was 41% on 23 July when the aphid population was greatest and rose to 55% in the next sample on 27 July when the aphid population had diminished by 90%. Soon afterwards, there were too few aphids to sample. The lucerne was drilled in April and more samples will be taken from the crop next year.

Germination of conidia. 83, 71, 26, 0 and 0% of conidia of *E. fresenii*, and 39, 5, 2, 0 and 0% of those of *E. aphidis* germinated on glass slides within 7 hours after discharge from infected aphids at 100, 97·5, 95, 90 and 85% relative humidity respectively, at 20°C in darkness. In the light fewer spores of both species germinated at each humidity. 49, 2, 0, 0, and 0% of conidia of *E. coronata*, discharged from cultures on artificial media, germinated on glass slides at the same range of humidities at 20°C in the light. Clearly *Entomophthora* conidia germinate only in saturated or near-saturated air, so the early morning when the air is moist is probably when most aphids become infected.

Resting spore formation and germination in *E. fresenii*. A culture of *E. fresenii* has been maintained on the bean aphid (*Aphis fabae*) in a glasshouse where the minimum temperature was 18°C and the day length is 18 hours from September to April. In September, 1969, and September, 1970, the culture was divided; half was retained in the glasshouse and half transferred out-of-doors to a shelter subject to ambient temperature and day length. Within a few aphid generations, resting spores always developed in some of the infected aphids in the shelter, whereas conidiophores continued to develop in all the infected aphids in the glasshouse. Some of the aphids that contained resting spores in 1969 were held in soil, which was occasionally moistened, in the shelter during the winter. They were brought into the laboratory, at about 20°C, in March 1970, and many of the resting spores produced conidia on long, slender conidiophores. These were identical to the characteristic secondary conidia of *E. fresenii*, that are produced on slender conidiophores from the primary conidia and are probably the primary infective form of the fungus. When healthy *A. fabae* were put in a container with germinating resting spores, many were killed by the fungus which produced conidiophores. The life cycle of an *Entomophthora* species has not previously been followed completely. Resting spores produced in September 1970, and brought into the laboratory at 20°C in October soon started to germinate, suggesting that increased temperature is all they need for germination. (Wilding)

Bumblebees. Pollen loads from bumblebees foraging on red clover (*Trifolium pratense*) contained acute bee paralysis virus, but not chronic paralysis virus. Also the pollen and anthers of the clover were free from acute paralysis virus and, as honeybees were not visiting the clover, it seems that bumblebees resemble honeybees in being inapparently infected and excreting the virus from their salivary glands into the fluid they add to pollen as they collect it. This agrees with previous results when bumblebees injected with extracts of seemingly healthy ones died and then contained many particles of acute paralysis virus. (Bailey)

Staff

Barbara Stanley and Mrs. Catharina G. Koster were appointed, and Sally A. Jennings, A. W. Raw and Doreen Watler left. W. J. Awram was awarded the Ph.D. degree of London University and returned to Canada. W. Fernando (University of Ceylon) joined as a temporary worker and P. J. Naylor (Hatfield Polytechnic) worked as a sandwich-course student.

N. Wilding attended the IVth International Colloquium on Insect Pathology in Maryland, U.S.A., and a meeting of the I.B.P. Working Party on 'Biological Control of Aphids' in Paris.