ROTHAMSTED RESEARCH

Report for 1968 - Part 1

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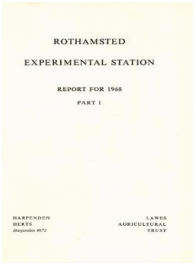

Bee Department

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C. G. Butler (1969) *Bee Department ;* Report For 1968 - Part 1, pp 223 - 232 **- DOI: https://doi.org/10.23637/ERADOC-1-123**

BEE DEPARTMENT

C. G. BUTLER

The department studies the behaviour, ecology, physiology and pathology of bees with the object of improving methods of beekeeping, making them cheaper and of finding the best ways of using honeybees, and possibly other insects, as pollinators of crops. Some results have implications extending beyond beekeeping and pollination. For example, studies of the ways in which behaviour is controlled by pheromones (i.e. chemicals, such as sex attractants, which are produced by one insect and influence another) may help in developing new methods for rapidly surveying large areas of crops for insect pests and controlling them by a more discriminating use of insecticides. Similarly, the work on pathology, which has been extended to some other insects than honeybees, is relevant to the use of pathogens to control insect pests.

The prolonged period of poor weather greatly hindered field work. It is hoped that the newly completed flight room, in which the temperature and humidity can be kept equal to those of summer days and nights, and with light enough for foraging, will enable some kinds of work with active colonies to be continued throughout the year. A new laboratory and glasshouse for insect pathology were also built.

Behaviour and physiology

Queen piping. The production of shrill sounds (piping) by young queen honeybees precedes the issue of swarms and may well be an important factor in determining whether a colony will actually emit a swarm. Individual young queens differ greatly in their readiness to pipe and different batches of queens, kept under the same conditions, were found to differ significantly in the amount they pipe. An apparent interaction between the time of year when queens were reared and their parentage suggested the existence of some factor that affected piping tendency and had not been completely equalised between groups and between times. Another experiment showed that queens reared from 0–1-day-old larvae were more inclined to pipe than queens reared from older larvae. (Simpson and Cherry)

The hive space colonies need. The combs occupied by bees in colonies with more than sufficient hive space were counted and the number of adult bees on them estimated by weighing, to find the mean number, which was about 1100 bees per British comb (35·5 × 21·6 cm, including frame), i.e. 1400 per Langstroth comb (44·8 × 23·2 cm). Using this mean, 81 colonies examined weekly throughout a season had maximum sizes ranging from 10 to 54 thousand bees, with a mean of 27 thousand. These and other observations suggest that, on average, colonies need the equivalent of at least 3½ British 11-comb boxes or 3 Langstroth 10-comb boxes to accommodate their adult bees. (Simpson)

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Winter survival of colonies. In very cold weather, the temperature among the bees at the surface of their cluster can be as cold as 10° C. Although these bees can only move sluggishly when disturbed, and are evidently chilled, colonies can survive long periods of cold weather without many bees dying. However, when single bees were kept in very small cages their lives were shortened by all temperatures from 30° C downwards, and at 20° C or colder had a mean length of only 4–5 days. At 20° C, bees caged in pairs ate less food, and had a greater expectation of life, than single bees. (Simpson)

Use of smoke to subdue bees. Beekeepers frequently use smoke to subdue (i.e. make less aggressive) bees when they open the hives. When bees are smoked in this way they eat honey from their combs. The average weight of the honeystomachs of bees was greatest ten minutes after smoking and then diminished, but even 24 hours later was more than before smoking. As bees that stung a provocative object had less food in their honey-stomachs than those that did not sting, a beekeeper will probably gain the full effect of the bees gorging, and be least liable to be stung, by delaying opening the hive until about ten minutes after smoking. However, only about half the bees gorge when their colony is thoroughly smoked, so other effects of smoke are probably important in inhibiting stinging. (Free)

Denatured sucrose for feeding bees. Sucrose with bitter tasting and coloured materials added, to make it unpalatable and unattractive for human consumption, is allowed by the Finance Act of 1968 to be sold without surcharge for use in animal foods. Approved mixtures, containing either sucrose octo-acetate ('Octosan') or benzyldiethyl ammonium benzoate ('Bitrex'), at 0·005% and 0·0005% respectively, in sucrose, each with the dye 'Green S' at 0·002%, were tested on bees. Young adult bees kept in cages at 30° C were fed concentrated solutions of sucrose containing these materials at the approved concentrations, or at ten times this concentration, in addition to water and pollen. The average time taken for half the bees to die ranged from 54 to 68 days, not significantly different from the 62 days taken by bees fed plain food. It seems unlikely, therefore, that the additives will have any harmful effect on bee colonies. However, some colonies were fed sucrose denatured with 'Octosan' + dye in autumn 1968 and their progress will be compared with the remainder overwintering on plain foods. 'Octosan' will probably be preferred to 'Bitrex' by beekeepers because it soon decomposes spontaneously in solution and traces that might eventually get into honey will, therefore, be tasteless. (Bailey)

Pheromones of queen honeybees. When virgin queens were introduced into the hives of colonies headed by mated, laying queens, some of the workers quickly formed aggressive 'balls' round the virgin queens and attempted to sting them to death. Occasionally, soon after a virgin queen had been introduced in this way, supernumerary 'balls' of aggressive workers formed without any queens in them and some workers were stung.

Experiments were made in which groups of workers were taken from colonies headed by mated, laying queens and caged for ten minutes either

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with live, or freshly killed, virgin queens, or rubbed against the bodies of such queens, and were then returned individually to their parent colonies. The behaviour of the parent colony towards them was observed and compared with that towards workers that (1) had been caged with strange mated queens, or rubbed against such queens, or caged without queens; (2) had been caged with virgin or mated queens but prevented from touching them although sharing the same atmosphere. All workers that had been in contact with virgin queens were roughly handled and some were dragged out of the hive whereas others were attacked and stung; a few were surrounded by 'balls' of angry bees. None of the other bees was stung; a few were lightly mauled, but most were only briefly examined when returned to their hives. As contact with the bodies of virgin queens was needed to arouse the aggressive behaviour of bees of colonies with mated, laying queens, their bodies presumably carry some substance not carried by mated, laying queens. Attempts are being made in collaboration with R. K. Callow (Insecticides Department) to identify this pheromone, which seems to occur on all major parts of a virgin queen's body. (Butler)

Work continued on the pheromone by which mated queens attract worker bees in the hive. (Butler, Callow, Insecticides Department, and Watler)

Pheromones of worker honeybees. When a bee stings an intruder, it releases pheromones from both a fold near the base of its sting and its mandibular glands. These mark the intruder and direct the attacks of other bees towards it. Iso-amyl acetate was identified in the sting pheromone and 2-heptanone in the mandibular-gland contents. Whether these substances provoke stinging was tested. The contents of crushed mandibular glands were no more effective than 2-heptanone in releasing stinging, and iso-amyl acetate was less effective than natural stings, so is not the only active component concerned. The sting venom itself had little or no effect. (Free and Simpson)

When bees are foraging at a rich source of food, such as a dish of sugar syrup, they sometimes expose their Nassanoff glands when flying over the food and when they begin to feed. Searching bees are attracted by the scent released from the Nassanoff glands and encouraged to alight. Observation of foraging bees showed that they usually made several visits to a dish (mean = 3·5) before exposing their Nassanoff glands, but individuals differed greatly. Once a bee had scented she continued to do so on about 90% of the remaining trips she made that day. Bees did not scent more freely when collecting honey than when collecting syrup, even when bees from strange colonies were present, so evolution of scenting behaviour is probably not connected with the robbing of other colonies of their honey stores. Neither the presence of Nassanoff gland odour, nor the presence of prominent visual orientation marks at a dish, influenced the tendency of bees to release scent, but they did so less often at dishes with a strong floral scent than without. However, even when foraging on natural flowers some bees exposed their scent glands. (Free)

Gas chromatography of freshly collected Nassanoff gland secretion confirmed that it contained nerolic and geranic acids, and citral. Strong

https://doi.org/10.23637/ERADOC-1-123

evidence was obtained that citral occurred in the fresh secretion and was not produced by oxidation of geraniol. Citral was much the most attractive component of the secretion to field bees, and a mixture of citral and geraniol (about 2·6:1·0 by weight) was almost as attractive as the natural secretion. (Butler and Calam, Insecticides Department)

Pollination and field behaviour

Effect of colony size on foraging activity. The hives of colonies in the same apiary were fitted with pollen traps and the pollen collected in each trap was weighed on several consecutive days to measure the relative foraging activity of each colony. On each of the four occasions the comparison was made, a deterioration in the weather discouraged foraging more from large then from small colonies. The ratio of brood to bees is larger in small than in large colonies and, because brood stimulates foraging, presumably it is usual for a larger proportion of the adult population of small colonies to go foraging. Consequently, when the weather improves, a small colony has less scope than a large one to increase the foraging proportion of its population. Although large colonies have more foragers at all times and so are to be preferred for pollinating crops, small colonies are probably more effective than their size would imply, especially when conditions for foraging are poor. (Free and Preece, Statistics Department)

Decreasing competition for pollinators. More bees from a colony visit a crop that needs pollinating when the colony is taken to the crop after it starts to flower, not before, because when taken before, the bees become accustomed to visiting other kinds of flowers, which they do not readily forsake. Conditioning to a crop needing pollination can also be helped by making use of the fact that flowers of some different species present most of their pollen at different times of day; for example, dandelion presents most of its pollen during the morning whereas apple does so during the afternoon. Not releasing bees from colonies taken to apple orchards until midday increased the proportion of apple pollen and decreased the proportion of dandelion pollen they collected; which should favour apple pollination, because dandelion is a serious competitor for bee visits. (Free and Nuttall)

Oilseed rape. The amount of oilseed rape (*Brassica napus*) grown in Britain has increased recently and the behaviour of bees on the crop was studied. All the honeybees observed collected nectar and none collected pollen only; however, they inadvertently became dusted with pollen as they brushed against the anthers and some packed this into their pollen-baskets whereas others discarded it. As the bees also touched the stigmas of 76% of the flowers they visited, they were effective in transferring pollen. Despite this, plots of rape caged with bees did not yield appreciably more seed than those caged without bees, indicating that automatic self-pollination is usual and that hiring honeybee colonies to pollinate rape is of doubtful economic value. (Free and Nuttall)

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Pollination of runner beans (*Phaseolus multiflorus*). Although runner bean flowers require insect pollination, bees fail to pollinate them during many of their visits. Most specimens of the bumblebee species *Bombus lucorum* and *B. terrestris* foraging on runner beans obtained nectar through holes they had bitten at the bases of the corolla tubes, and although honeybees could not make these holes, 80% of those present obtained nectar through them. The appearance of such 'robber' bumblebees was quickly followed by an increase in the honeybee population, but after the bumblebees had disappeared for the season and further holes were not being bitten, many of the 'robber' honeybees changed to entering the mouths of the flowers when seeking nectar. Therefore the behaviour of 'robber' bumblebees may even be advantageous because they attract to the crop honeybees that eventually enter the flowers and pollinate them. Few of the honeybees that entered the flowers had pollen loads, but workers of the bumblebee, *Bombus agrorum*, always entered the flowers and all of them collected pollen. Even so, tests showed that honeybees were as effective as bumblebees at pollinating runner beans in a glasshouse, and more so than blowflies, and so can be used to produce earlier and more profitable crops. In a glasshouse without 'robber' bumblebees, honeybees can obtain nectar only by entering the flowers, so their pollinating efficiency per flower visit is likely to be much greater than in the field. (Free)

Foraging behaviour of wasps. Worker wasps (*Vespula germanica* and *V. vulgaris*) preying on honeybees preferred pupal honeybees, perhaps because their cuticle was softer, to adults, and newly emerged adults to older ones. Nevertheless some individuals continued to prey on adult bees even when they could have chosen pupae. Before flying home with their booty, the wasps always severed the bodies of adult bees at the neck or waist, whereas pupae were severed at various places. Individual wasps did not follow a regular pattern when dismembering adult bees but always collected the largest loads they could carry. During the autumn, when these observations were made, the abdomen and thorax of a bee were equally attractive to a wasp and preferred to its head.

Most wasps that were preying on bees (i.e. collecting protein) could easily be persuaded to collect sugar syrup instead, but the reverse was much more difficult to achieve.

Wasps were often attracted to a site by seeing others visiting it, but some that had found food tried to repel others that attempted to join them. The frequency with which a wasp returned to a site depended on how often it had previously found food there. (Free)

Bee diseases and pests

Chronic paralysis. Of 12 colonies headed by queens reared in 1967 from larvae from colonies severely affected by paralysis, two became severely affected in 1968, whereas none of the many colonies headed by queens bred from healthy colonies was affected. This difference is highly significant ($P < 0{\cdot}001$) and confirms that susceptibility to the multiplication of chronic paralysis virus is closely linked with hereditary factors. A daughter of a

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queen that died of paralysis soon after she laid her first eggs in 1967 headed one of the two severely affected colonies that seemed healthy until mid-June, 1968. The other severely diseased colony had become much larger than average after being given the new queen early in July 1967, and had collected much surplus honey by the end of the year, but it collapsed within two weeks in May 1968, to a handful of bees, with many thousands of individuals crawling and dying on the ground. Such striking effects of disease have not been seen to be caused by any pathogen other than chronic paralysis virus.

Queens were successfully reared in 1968 from larvae taken from a colony while it was severely affected with paralysis, and twelve were successfully mated. Two disappeared later and one found dead was infected with chronic paralysis virus. The others head colonies that continue to be unaffected.

Immature pupae and newly emerged moribund worker bees that were collected from beneath colonies with paralysis contained much chronic paralysis virus. This suggested that they had become infected while they were larvae. However, larvae taken from colonies suffering from paralysis and inoculated with chronic paralysis virus produced adults that remained apparently healthy in the laboratory. Newly emerged adults were obtained from colonies with severe paralysis by incubating mature pupae in the laboratory. Usually these adults remained apparently healthy and seemed not especially susceptible when fed with chronic paralysis virus. However, on one occasion about half the newly emerged bees that had been left for a few hours on their comb became paralysed after a few days in cages, whereas further bees, caged as soon as they emerged from the same comb, remained healthy. This suggests that bees become infected from the comb.

Many live paralytic workers in badly affected colonies are unusually bloated with honey, each having about 30 mg in her honeystomach. Infectivity tests showed that this honey contained about 10^{12} particles/ml (10^{10} LD_{50s} by injection) of chronic paralysis virus. Bees may pass infection to others by regurgitating their food, possibly into cells near emerging brood.

Paralysed drones, and immature drone pupae collected from beneath colonies with paralysis, contained as much chronic paralysis virus as workers and, as in workers, most virus was in their heads. This suggests that the large glands in the heads of workers do not harbour much virus because these glands are atrophied or lacking in drones, and agrees with infectivity tests that showed most virus in paralysed workers to be in their brains.

Acute paralysis. Early experiments suggested that acute paralysis virus, which is common in apparently healthy bees, could be activated by injecting such bees with plant viruses or some other materials, causing death from acute paralysis. Attempts to repeat these results by injecting bees with turnip yellows mosaic virus from various sources including Rothamsted (freshly prepared by Varma, Plant Pathology Department) failed. It must be assumed, therefore, that the preparations made earlier were contaminated with acute paralysis virus. This has also been suggested by

https://doi.org/10.23637/ERADOC-1-123

results with sacbrood virus. Bees injected with preparations of sacbrood usually died of acute paralysis and it was assumed that this virus had been activated by the injected sacbrood virus. However, on several occasions acute paralysis virus failed to multiply in bees that were injected with one particular preparation of sacbrood virus. In view of this and the results obtained with the plant viruses, it seems probable that sacbrood virus preparations are almost always contaminated with acute paralysis virus. This is not surprising, because acute paralysis virus is not only common in honeybee colonies, but also is the same size and sediments at the same rate as sacbrood virus, so cannot be physically separated from it. However, these viruses are serologically different, which has enabled further progress to be made on the multiplication of sacbrood virus in adult bees.

Sacbrood. Many experiments in which sacbrood virus was injected into adult bees failed to give any evidence that it multiplied, but this now seems probably because acute paralysis virus, which multiplied in the injected bees, interfered with the multiplication of sacbrood virus. This was indicated by the fact that a few larvae developed sacbrood when fed with extracts of adult bees that had been injected with a sacbrood virus preparation (see above) seemingly uncontaminated with acute paralysis virus. Further tests were made with bees injected with both sacbrood virus plus rabbit antiserum prepared against acute paralysis virus. This antiserum prevented acute paralysis virus from multiplying in the bees, and extracts of these, especially of their heads, then caused sacbrood when fed to larvae. The extract of the head of one injected bee contained about $10^2 LD_{50s}$ of sacbrood virus when given in food to larvae and the least infective dose for adults by injection was about 10^{-4} of an LD_{50} in food for larvae. Much sacbrood virus was in the hypopharyngeal glands of infected adults. Adult bees younger than about four days were infected when they ingested sacbrood virus. The least infective dose for them by mouth was about $10^2 LD_{50s}$ for larvae. Adult bees infected with sacbrood virus either by feeding or injection showed no symptoms. However, young adults ate no more pollen after ingesting sacbrood virus. When they were infected before they had eaten any pollen they lived in the laboratory only as long as uninfected bees that were not supplied pollen, about three weeks, whereas uninfected bees supplied ample pollen lived about nine weeks. Young bees injected with sacbrood virus also ate no more pollen, whereas bees injected with water continued to eat pollen and live normally. Bees had to be immobilised by chilling (about 20 minutes at 4°) to be injected in these tests because CO_2 anaesthesia, which is usually employed, also stopped bees from eating more pollen. Thus, although bees appear unaffected by infection with sacbrood virus, their lives are considerably shortened and their behaviour is probably changed, perhaps in a way similar to that in which it is known to be changed by CO_2 anaesthesia.

Young bees were also successfully infected after they had eaten almost enough pollen to live a usual length of life. Bees of this kind probably form a reservoir of sacbrood virus in honeybee colonies. (Bailey)

https://doi.org/10.23637/ERADOC-1-123

Pathology of other insects

Entomophthoraceae. The orange-coloured, aculeate resting spores found infecting Wheat Bulb flies (*Rothamsted Report for 1967*, p. 218) were identified as *Tarichium hylemyiae* Lakon.

Wheat Bulb flies were found with cysts occupying much of their abdomens. Each cyst is lined internally with a sporulating fungus, the spores of which are discharged through a smoothly rounded opening on the sternum of the fly. This fungus was identified as *Strongwellsea castrans* Bakto, which, as *T. hylemyiae*, is associated only with hylemyid flies.

About 100 Wheat Bulb flies per week were collected from the beginning of July to the middle of August at White Horse 1 field, to estimate, as in 1967, the incidence of fungi of the family Entomophthoraceae. To prevent spread of infection, the flies were kept singly in cages in an insectary. After two weeks, by when most flies infected with Entomophthoraceae would have died, they were put together in larger cages. Flies were examined for the fungi when they died. Only one infected fly, a female killed by *Entomophthora muscae* on 24 August, nine days after capture, was found. Other observers also found very few infected flies in 1968.

Although *E. muscae* was consistently transmitted from infected scatophagid flies to healthy ones, it was not transmitted, under identical conditions, from infected scatophagids to Wheat Bulb flies.

Samples of 100 pea aphids (*Acyrthosiphon pisum*) on lucerne were taken from two sites on Highfield on eight occasions and on peas in the Garden Plots in one occasion, to estimate the incidence of *Entomophthora* spp. Regular sampling from the lucerne was impossible because aphids could not be collected for about two weeks after each cutting of the lucerne and because few aphids were found after potatoes on adjacent sites had been sprayed with 'Metasystox' on 19 July. Table 1 shows the percentages of infected aphids. They were similar in two-year-old and one-year-old crops until 20 May, after when the younger crop, as in October 1967, had more.

Table 2 shows the incidence of *Entomophthora* spp. in the black bean aphid (*Aphis fabae*) sampled weekly from field beans in the Garden Plots from 23 August, when the aphids first appeared, until they disappeared a month later.

An additional 19–30% of bean aphids were found parasitised by Hymenoptera on each occasion.

TABLE 1

Incidence of Entomophthora *spp. on aphids on* (*a*) *lucerne and* (*b*) *peas*

	Sampling date	% infected* with *E. aphidis*	% infected* with *E. thaxteriana*
(a)	14 May 1968	0	23
	20 May 1968	0	55
	27 May 1968	0	22
	17 June 1968	2	0
	26 June 1968	1	21
	1 July 1968	0	18
	15 July 1968	45	36
(b)	15 July 1968	29	23

* The two spp. of *Entomophthora* were not found together on individual aphids.

https://doi.org/10.23637/ERADOC-1-123

TABLE 2

Incidence of Entomophthora *spp. on bean aphids*

Sampling date	% infected* with *E. aphidis*	% infected* with *E. planchoniana*
23 July 1968	16	1
29 July 1968	14	4
5 August 1968	24	9
12 August 1968	7	7

* The two spp. of *Entomophthora* were not found together on individual aphids.

The susceptibilities of the aphids, *A. pisum*, *A. fabae*, *Myzus persicae*, *Cavariella theobaldi* and *Sitobion fragariae* to *Entomphthora thaxteriana*, *E. aphidis* and *E. fresenii* were tested. *E. thaxteriana* was readily transmitted from infected *A. pisum* to each of the other aphid species, and from them back to *A. pisum*. *M. persicae*, *C. theobaldi* and especially *S. fragariae* were less susceptible than *A. pisum* or *A. fabae* to *E. aphidis*. *E. fresenii* was consistently transmitted to *A. fabae* but to very few individuals of the other species, though it was readily transmitted back to *A. fabae*. Thus, of the three species, *E. fresenii* seems the most host specific and *E. thaxteriana* the least. There was no evidence that there were strains, within a species of *Entomophthora*, best adapted to different host species. For example, *E. fresenii* was as readily transmitted from the few individuals of *A. pisum* that could be infected, to *A. fabae*, as it was from *A. fabae* to *A. fabae*. Similarly, it was no more readily transmitted from *A. pisum* to *A. pisum* than it was from *A. fabae* to *A. pisum*.

Preliminary tests show that *C. theobaldi* and *A. fabae* are more susceptible than *A. pisum* to *E. planchoniana*. Attempts to transmit *E. virulenta*, which was found infecting aphids in a laboratory culture of *C. theobaldi*, failed.

Neither *E. thaxteriana* nor *E. aphidis* could be transmitted from infected *A. pisum* to the Delphacid, *Delphacodes pellucida*.

E. aphidis and *E. virulenta* were isolated on sterile egg yolk. *E. aphidis* was also grown on a yeast/starch/milk powder agar and *E. virulenta* on a yeast/starch/peptone agar. *A. pisum*, *A. fabae* and *M. persicae* were infected with conidia from *E. aphidis* cultured on agar.

At 100% relative humidity and at room temperature, *E. thaxteriana* on infected pea aphids began sporulating about ten hours after an aphid died, and sporulation reached a maximum about eight hours later whether in darkness or light. Sporulation almost ended 36 hours after an infected aphid died and ended after a further 30 hours. Cooling prolonged sporulation and slowed the production of conidia. An average of $3{\cdot}6 \times 10^4$ conidia were produced on one apterous adult aphid.

The fungus within aphids infected with *E. thaxteriana*, *E. aphidis* and *E. fresenii* remained viable for some weeks when the insects were taken from the plant and dried soon after they died. When the dried insects were moistened the fungus soon began to produce infective conidia. *E. aphidis* on *A. pisum* sporulated after being kept for 31 or more days at 20% or 40% relative humidity, and after 28 days, but not 31 days, at 60% relative humidity. It survived for fewer than 21 days at 80%, and the dead aphids became overgrown with saprophytic organisms. (Wilding)

https://doi.org/10.23637/ERADOC-1-123

Staff

D. Boothroyd, Sarah Cherry, P. M. Nuttall and W. A. Stevens left and T. Scott-Hibbert, Ellen Moxley, Clare I. Pearce and Ingrid H. Weinberg were appointed.

https://doi.org/10.23637/ERADOC-1-123